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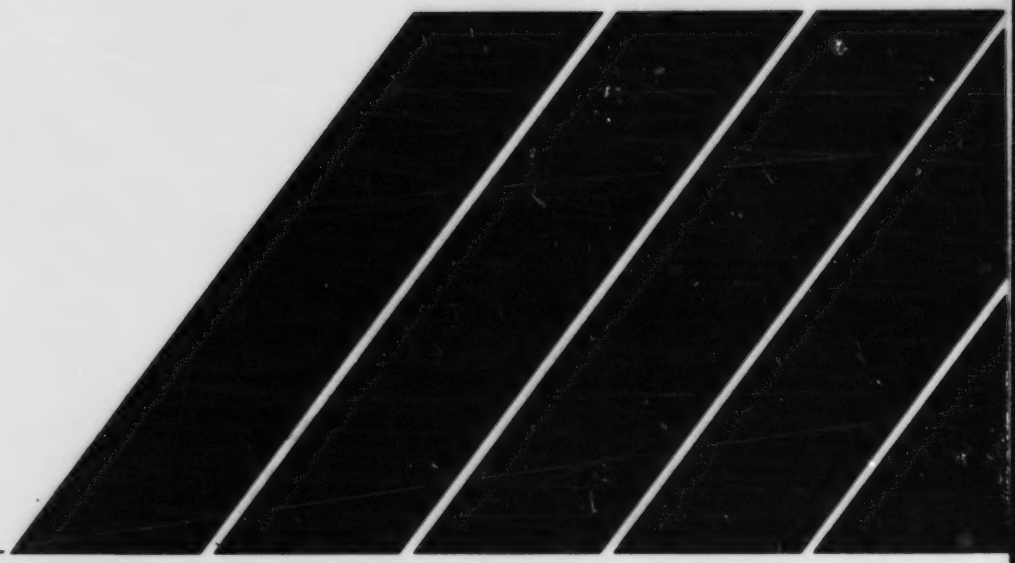
PRODUCTION OF ETHANOL, PULP FIBRE AND ANIMAL FEED FROM LOW GRADE HARDWOODS



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PRODUCTION OF ETHANOL, PULP FIBRE AND ANIMAL FEED FROM LOW GRADE HARDWOODS

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PREFACE

The "Centre de recherche en sylvichimie de l'Outaouais" (CRSO) is a research centre dedicated to the development and promotion of new technologies for the transformation of wood wastes into marketable products. This report summarizes the research results covering the period from December 1, 1987 to August 1, 1990 on the development of new technologies for the production of pulp from low grade hardwoods based on the CRSO nitric acid process and the utilization of sugars in the waste waters and hydrolysed fines fibres for the manufacture of ethanol and animal feed. This research has led to a patent application for the CRSO process, a copy of which is included.

ABSTRACT

The utilization of hardwoods is, at present, very limited because most vessels and ray (parenchyma) cells are very short and not valuable for paper-making. In a new catalyzed nitric acid hardwood pulping process developed by the CRSO (patent pending), the long fibre fraction is recovered, bleached and used to produce a good quality paper pulp. About 70% of the wood fibre can be recovered for sale as pulp, with the remaining 30% as short fibres (fines) used to produce ethanol.

The pulping process does not require expensive high temperature and pressure equipment because both the acid concentration and pulping temperature are relatively low and the process takes place at atmospheric pressure. The process is environmentally friendly since it is possible to bleach the pulp fibres without using chlorine, consequently avoiding the production of environmental hazards such as dioxins. In addition, options exist for recovering used acid and ammonia bleaching agent for the production of either microbial protein for animal feed or as an ammonium nitrate fertilizer.

The short fibre fraction of the wood is enzymatically hydrolysed to liberate the wood sugars which are simultaneously fermented by yeasts to produce ethanol. The sugars left in the used acid and wash waters are then used as a substrate for the growth of an acid-tolerant yeast-like fungus, *Scytalidium acidophilum*, thereby producing fungal protein animal feed supplement, while removing the biochemical oxygen demand (BOD) of the waste water.

Pulp and ethanol production data and pulp quality data from both bench scale and pilot plant is presented. A preliminary economic assessment is included.

RÉSUMÉ

Les bois feuillus sont actuellement sous-utilisés car une grande partie de leurs vaisseaux et cellules ray (parenchyme) sont trop courtes et ont peu de valeur pour la fabrication de pâte à papier. Dans le nouveau procédé de mise en pâte à l'acide nitrique en présence d'un catalyseur, développé par le CRSO (brevet en suspens), les fibres longues sont séparées des fibres courtes pour être ensuite blanchies et utilisées pour la production de pâte à papier de bonne qualité. Environ 70% des fibres peuvent être ainsi utilisées pour la pâte et le 30% des fibres courtes restantes est utilisé pour la production d'éthanol.

Ce procédé de mise en pâte ne nécessite pas d'équipement coûteux pouvant résister à de hautes températures et pressions car les températures de cuisson sont relativement basses et se font à la pression atmosphérique. Il s'agit d'un procédé qui est environnementallement sain, le blanchiment de la pâte se faisant sans utilisation de chlore, évitant ainsi la production de composés nocifs tels les dioxines. De plus, la réutilisation des acides et des agents de blanchiment dans le procédé, ainsi que la possibilité d'utiliser les effluents pour la production de protéines unicellulaires servant de nourriture pour les animaux ou pour la production de fertilisant nitraté (amendements de sol) demeurent des options.

La fraction des fibres courtes est hydrolysée enzymatiquement afin de produire des sucres qui sont simultanément fermentés, par des levures, pour produire de l'éthanol. Les sucres résiduaires présents dans l'acide usé et les eaux useés peuvent servir de substrats pour la croissance de microorganismes pouvant tolérer un milieu acide (*Scytalidium acidophilum*), produisant ainsi des protéines d'organismes unicellulaires et réduisant par le fait même la consommation d'oxygène (D.B.O.) dans les effluents.

Les paramètres d'opération pour la production de la pâte et de l'éthanol ainsi que l'évaluation de la qualité de la pâte provenant à la fois du laboratoire et de l'usine pilote sont présentés. Une évaluation économique préliminaire est aussi incluse.

EXECUTIVE SUMMARY

Canada's forests contain high value softwoods, utilized for lumber and paper, and hardwoods, of which only a few species have commercial value for lumber or paper pulp. Relatively large volumes of poplar and white birch are still available in the Outaouais and other regions of Québec for pulp and paper manufacture or for ethanol production. Because some hardwood fibres are longer than the average length of softwood-groundwood fibres used for making newsprint, separation of the wood fibres from the short vessels would produce a very useful and valuable paper pulp.

What is described in this report are the research results that led to a patent application (Appendix 1) for a novel dilute nitric acid pulping process, referred to here as the CRSO process. This process involves mild nitric acid pulping of hardwoods, in the presence of a phosphoric acid catalyst, at temperatures below 100°C at atmospheric pressure to produce a pulp that can be separated into long fibre fraction for sale as paper pulp, and a short fibre (fines) fraction for hydrolysis to glucose and subsequent fermentation to ethanol.

This pulping process proved to be very successful, and since it also rendered the short fibre fines to be nearly 100% accessible to enzymatic hydrolysis, it was decided to use a simultaneous enzymatic hydrolysis and fermentation (SHF) approach to produce ethanol from the short fibre fraction of the pulped wood. The SHF approach was selected because initial studies using a secondary acid hydrolysis of the wood fines produced very low yields of glucose for fermentation.

The pulping variables studied included: nitric acid concentration, phosphoric acid concentration, cooking temperature, cooking time and wood to liquor ratio. The effects of these independent variables were tested against the following dependent variables: yield of pulped wood, refining energy needed to fiberize the cooked chips, freeness of the pulp (a paper making quality), and pulp fibre physical properties as used in the pulp and paper industry.

The base case pulping conditions selected were: 3 hour cooking of wood chips at 95°C in a liquor consisting of 5.0 wt% nitric acid (HNO_3) and 0.25 wt% phosphoric acid (H_3PO_4). Wood dry weight to cooking liquor ratio was 1:4.

Overall, the results of pulp characterization indicated that the tear index of paper made with 100% CRSO pulp may be too low for use as newsprint furnish, but replacing kraft pulp in newsprint furnish with an optimized birch CRSO pulp is definitely feasible and there should be a ready market for this pulp.

The pulp produced can be used in the unbleached state or it can be bleached to a higher degree of whiteness by extraction with sodium hydroxide or ammonium hydroxide. Significant increases in brightness from 24.6% for unbleached pulp to 69.6% (NaOH) and 57.5% (NH_4OH) were observed following only one extraction. This is a significant finding in light of the growing concern over the environmental hazards associated with chlorine bleaching of kraft pulp.

The simultaneous hydrolysis and fermentation (SHF) studies showed that at an initial substrate concentration of 10 to 15 wt%, SHF over 48 to 120 hours, allowed 90% cellulose hydrolysis and 73% of the theoretical ethanol yield. These results confirmed that the CRSO nitric acid pulping process renders the cellulose in the pulp fines very susceptible to enzymatic hydrolysis.

During the pulping reaction there are gases produced. The composition of the process gas is not completely known. The components identified included various oxides of nitrogen (NO_x) and hydrogen cyanide (HCN). A properly designed gas incinerator and gas scrubbing system can be designed to treat the reaction gases. Theoretically, after ignition the HCN and NO can be completely oxidized to NO_2, which can then be absorbed in water to form nitric acid. Demonstration tests of this proposed acid recovery system remain to be carried out. Nitric acid recovered in this manner can be reused in the cooking liquor.

Because of the acute toxicity of cyanide, additional studies were carried out to determine ways of minimizing HCN in the reaction gas, used cooking liquor, and wash water. After adjusting condensate from the pulping reaction gases to

pH 10 to facilitate HCN oxidation by hydrogen peroxide, cyanide analysis results confirmed that with sufficient hydrogen peroxide present, total HCN oxidation could be achieved. Less than 2 ppm cyanide was present in the fermentation beer prior to distillation. The addition of 100 ppm hydrogen peroxide was effective in reducing this level to <1 ppm. Hydrogen peroxide treatment of process gas condensate should not be necessary if proper gas incineration and scrubbing is used.

The chip wash water, refiner water and water used in the separation of the long and short fibre fractions all contain various amounts of sugars, primarily xylose, that contribute to the biochemical oxygen demand (BOD) of this waste stream. In order to reduce BOD, these waste waters were collected and treated in an up-flow column reactor containing mycelium of *Scytalidium acidophilum*, an acid-tolerant, yeast-like fungus. Initial tests showed almost 50% removal of xylose and acetic acid, as well as a certain amount of the yellow colour in the waste water. With sufficient hydraulic residence time and an adequate bed of active mycelium, more complete removal of xylose and organic acids should be possible. Further work is required to optimize the used acid and water treatment process in order to produce sufficient microbial protein for sale as animal feed.

A series of eight pulping cooks were completed in the CRSO pilot plant. The results confirmed the bench scale findings, and the pulp produced was superior to our initial results. SHF results were less than expected, producing 50% of the theoretical ethanol yield, because the large SHF reactor vessel could not maintain the necessary anaerobic atmosphere required for an efficient fermentation. Several SHF improvements are still possible.

Preliminary economic evaluation of the CRSO process showed that a 600 odt wood per day pulp mill producing 27 million L/y of ethanol would be economically feasible at the existing CRSO Gatineau distillery site. The use of adjunct sources of cellulose from local wastes, such as pulp mill clarifier sludge or recycled waste paper, is recommended to produce sufficient ethanol to run the distillery at capacity.

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1. INTRODUCTION

Canada's forests contain high value softwoods, utilized for lumber and paper, and hardwoods, of which only a few species have commercial value for lumber or paper pulp. There is a large number of weed-tree species, such as grey birch, white birch, poplar and Manitoba maple, for which there is no commercial use, apart from waferboard manufacture, because of their short average fibre length. Relatively large volumes of poplar and white birch are still available in the Outaouais and other regions of Québec for pulp and paper manufacture. The only other possible use for these weed-tree species would be for energy (Ranney *et al.*, 1985).

Hardwoods consist of wood fibres, vessels and ray (parenchyma) cells. While the wood fibres are relatively long, most vessels and parenchyma cells are very short as shown in Table 1.

Table 1. Fibre Lengths of Some Softwoods and Hardwoods[1]

Source	Mean length of Fibres (mm)	Vessels (mm)
Softwood:		
Spruce (tracheid cells)	3.5	-
Hardwood:		
Birch	1.5	1.0
Aspen	1.1	1.0
Oak	1.3	0.25

(1) Adapted from Koran, 1989.

Since these hardwood fibres are longer than the average length of softwood-groundwood fibres used for making newsprint, usually 1.1 mm, separation of the wood fibres from the short vessels would produce a very useful and valuable paper pulp. There is a shortage of softwood for pulp and paper manufacture, and more hardwoods will have to be utilized for pulp production, as well as for lumber and particleboard. The utilization of hardwoods is at present

limited to about 10% of the available trees and new markets and new uses are being sought to further utilize these valuable reserves.

What is described in this report are the research results that led to a patent application for a novel dilute nitric acid pulping process, referred to here as the CRSO process. This process involves mild nitric acid pulping of hardwoods, in the presence of a phosphoric acid catalyst, at temperatures below 100°C at atmospheric pressure to produce a pulp that can be separated into long fibre fraction for sale as paper pulp, and a short fibre (fines) fraction that can be enzymatically hydrolysed to glucose and simultaneously fermented to produce ethanol.

The increasing demand for quality pulp and the huge potential market for ethanol for use in octane-enhancing, ethanol-methanol-gasoline blends for lead-free automotive fuels, suggests a ready market for the major products of the CRSO process. Other by-products, including animal feed supplements, compost soil conditioners, and ammonium nitrate fertilizers have potential markets as well.

A further introduction to nitric acid pulping and the use of the short fibre fraction for ethanol production can be found in the patent application in Appendix 1. For a detailed account of all aspects of conventional pulping processes, the reader should refer to the excellent book by Rydholm (Rydholm, 1985).

2. MATERIALS AND METHODS

2.1 CRSO pulping procedure

A patent application for the CRSO pulping process has been filed with the Canadian Patent Office. A copy of this patent application, presented in Appendix 1, describes the CRSO nitric acid pulping process in detail.

The majority of the studies on long fibre pulp properties and ethanol production from the fines used fibres produced under our base case pulping conditions, referred to in this report as Condition No. 8 (out of 40 different process conditions tested and described in Section 3.1.1). Commercial size white birch chips used in the pulping study were obtained from Tembec Inc., Temiscaming, Quebec.

The base case pulping conditions included: 3 hour cooking of wood chips at 95°C in a liquor consisting of 5.0 wt% nitric acid (HNO_3) and 0.25 wt% phosphoric acid (H_3PO_4). Wood dry weight to cooking liquor ratio was 1:4. Other pulping details, including bleaching and refining (fiberizing) are described with the results of these studies.

2.2 Estimate of cooked chip refining energy

An experimental stainless steel device developed by Dr. W. Kaeppner was used to estimate the refining energy, expressed in kilowatt-hours (kWh), required to fiberize the pulped wood chips. The refining energy was related to the number of turns of a cylindrical series of refiner blades required to fiberize the cooked chips so that they passed through the device. A constant weight plunger acted on the charge of cooked chips to force them onto the refiner blades. Water was passed through the device during refining to simulate the operation of a disk refiner.

2.3 Pulp characterization

Standard procedures of the Technical Section of the Canadian Pulp and Paper Association (CPPA) were generally followed for fibre treatment and physical testing of pulps. These included preparation of handsheets (C.4, 1950), Canadian Standard Freeness (C.1, 1972), bulking thickness (D.5, 1965),

bursting strength of paper (D.8, 1964), internal tearing resistance of pulp handsheet (D.9, 1977), and tensile breaking strength of paper (D.6, 1961). Optical properties tested included brightness of paper and opacity of paper, which were measured using a Technibrite Micro TB-1C (Technidyne Corp.).

2.4 Simultaneous hydrolysis and fermentation (SHF)

SHF experiments were carried out in 22 X 200 mm glass test tubes fitted with CO_2 water traps. Initial substrate loading was 5.0 g dry wt wood fines, adjusted to pH 4.5 - 4.8. Fresh cultures of commercial pressed bakers' yeast, *Saccharomyces cerevisiae*, produced by Lallemand Inc., Montreal, were obtained from a local bakery on the day each experiment was started. To an aqueous mixture of cellulase, cellobiase and occasionally protease, an inoculum of yeast was added to equal 6 dry wt% of the initial wood dry wt. The enzymes used are described in Section 3.4. The enzyme-yeast solution, adjusted to pH 4.5 - 4.8 was then added to each tube to commence SHF and incubated without shaking in a water bath at 30°C. Each tube was mixed daily by vortex. Total reaction volume was varied by addition of water to obtain different initial wood solids ranging from 10 to 21 wt%. No other nutrients were added. Several tubes were prepared using the same batch of enzyme-yeast mixture and duplicate tubes were harvested each day to determine the extent of SHF. Ethanol was analyzed by gas chromatography and residual sugars by HPLC using Bio-rad Aminex HPX-87P column. Dry weights were determined following drying at 105°C to constant weight.

2.5 Reaction gas analysis

A Miran 1B infrared spectrophotometer, used to detect organic vapours and HCN, an oxides of nitrogen analyzer and various detector tubes were used to determine the approximate composition of the reaction gas produced during pulping. This analysis was performed by personnel from the Industrial Hygiene Laboratory of Labour Canada, Ottawa.

2.6 Cyanide analysis and H_2O_2 oxidation

Cyanide in used cooking liquors, wash waters and condensates was analyzed by the CANMET Mineral Sciences Laboratory, Energy, Mines and Resources Canada, Ottawa, using their standard method (Sweileh, 1989).

In preliminary studies, hydrogen peroxide (H_2O_2) was added to cyanide-containing waste streams to oxidize cyanide to cyanate and finally carbon dioxide (CO_2) and nitrogen gas (N_2) (Hyatt, 1976). The wastes were adjusted to pH 10 with NaOH to ensure dissociation of the cyanide ion (CN^-) before addition of H_2O_2. Equimolar amounts of H_2O_2 and CN^- are needed for cyanide oxidation.

2.7 Growth of *Scytalidium acidophilum*

The acid tolerant fungus, *Scytalidium acidophilum* (ATCC 26774), can exist in either a yeast-like (single cell) form or as fungal mycelium. The yeast form is desirable for xylose fermentation. The mycelial form is used for growth on xylose and other sugars in acidic waste waters for the production of fungal protein as a potential animal feed.

The following medium was developed that allows for the growth of *Scyt. acidophilum* in the yeast form. The same medium was also used to grow the mycelial form after adjusting to pH 0.7.

Kaeppner's Medium

H_2O	1000 mL
$(NH_4)_2SO_4$	1.4 g
KH_2PO_4	2.0 g
$MgSO_4.7H_2O$	0.3 g
$CaCl_2.2H_2O$	0.3 g
Yeast extract	1.0 g
Urea	0.3 g
Essential Minerals	1.0 mL
Ivarson Nutrients	2.0 mL
Glucose or sucrose	38.0 g

Essential Minerals:	g/L	Ivarson Nutrients:	mg/L
$FeSO_4.7H_2O$	5.0	H_3BO_3	120
$MnSO_4.4H_2O$	2.0	$NaMoO_4.2H_2O$	500
$CoCl_2.2H_2O$	2.0	$CuSO_4.5H_2O$	800
$ZnCl_2$	1.8	$MnCl_2.4H_2O$	150

Adjusted to pH 4.5 - 4.7 with H_2SO_4.
Yeast extract and sugar were added just before inoculation.

2.8 CRSO pilot plant description

In order to apply bench scale (0.7 kg wood wet wt) findings on a larger scale, a pilot plant capable of handling 50 kg batches of wood wet wt was assembled. The pilot plant consisted of the following equipment:

The **reactor or digester** was a 135 L stainless steel, jacketed reactor that was heated by steam from a portable 11.2 kW oil-fired boiler. The reactor was fitted with a reflux (vertical) condenser. The cold water condenser exhaust was connected via a gas line to a gas incinerator outside of the building. The reactor was operated at atmospheric pressure. Inside the reactor the wood chips were placed in a perforated stainless steel basket, which allowed easy removal of the cooked wood chips. The reactor was also equipped with a bottom screen to allow it to be used for bleaching of the long fibre fraction. Fresh and used cooking acids were pumped to the reactor by a stainless steel pump. A vacuum pump was used to evacuate the reactor to 635 mm Hg for chip impregnation by the cooking liquor.

This reactor saw double duty as the **distillation** vessel. For distillation, the condenser was mounted horizontally from the top plate of the reactor.

The **refiner** was a Bauer Lab refiner equipped with standard refiner disk plates and powered by a 3.73 kW motor. The **fibre separator**, used to separate the long pulp fibre fraction from the short fines fraction, consisted of a vibrating slotted screen plate covered with an additional polyester 40 X 50 wires per inch mesh screen. This effectively separated the long fibre pulp equivalent to a standard R 80 sieve. This machine was also used with just the original slotted screen plate in place for de-shiving the pulp to remove the larger, uncooked wood chips (shives).

The **simultaneous hydrolysis and fermentation (SHF) reactor** was a temperature-controlled 300 L stainless steel horizontal tub in which a helical (double ribbon) impeller was mounted horizontally and turned at a constant speed of 4.5 rpm. The reactor was covered with a clear plexi-glass lid to allow observation of SHF progress. While the initial head space was purged with CO_2 at the start of each SHF, a proper seal and air trap arrangement was

unfortunately not maintained because the lid had to be opened to take representative samples of the high solids SHF mixture.

3. RESULTS AND DISCUSSION

3.1 Pulp fibre production using low grade hardwoods

3.1.1 Optimization of wood pulping

White birch chips were cooked in a mixture of nitric acid (HNO_3) and phosphoric acid (H_3PO_4) at temperatures from 65 to 95°C for 0.5 to 4.0 hours under atmospheric pressure. Consequently, high temperature and pressure vessels were not needed for this process.

Initially, our plan was to use the same acid for pulping and acid hydrolysis of the cellulose fines. Consequently, cooking parameters were selected to maximize the wood sugars (i.e., glucose, mannose, xylose, galactose, arabinose) found in the cooking liquor. These sugars would then be fermented along with the glucose from acid hydrolysed cellulose fines using the acid-tolerant fungus, *Scytalidium acidophilum*, to produce ethanol and microbial protein for animal feed. While there were some sugars liberated during the pulping step, acid hydrolysis of the cellulose to glucose was not effective for reasons yet unknown. Even the use of other dilute mineral acids (HCl and H_2SO_4) at elevated temperature and pressure could not produce adequate amounts of glucose from the cellulose fines obtained following our nitric acid pulping process. These results were confirmed by experiments carried out by Professor E. Chornet of the University of Sherbrooke and by Bio-Hol Developments Ltd. of Toronto.

The pulping step proved to be very successful and since it also rendered the short fibre fines to be 100% accessible to enzymatic hydrolysis, it was

decided to use a simultaneous enzymatic hydrolysis and fermentation (SHF) approach to produce ethanol from the short fibre fraction of the pulped wood.

The pulping variables were:

1. Nitric acid (HNO_3) concentration (0.5, 2.5, 5.0%);
2. Phosphoric acid (H_3PO_4) concentration (0, 0.25, 0.5%);
3. Cooking temperature (65, 80, 95°C);
4. Cooking time (2.5, 3, 4 hours);
5. Wood:Liquor Ratio (1:5, 1:4, 1:3).

The effects of these independent variables were tested against the following dependent variables:

1. Yield of pulped wood;
2. Refining energy needed to fiberize the pulped chips;
3. Freeness of the pulp;
4. Pulp fibre physical properties (for use in pulp).

A Box-Behnken factorial experimental design was originally planned to determine the independent variables most affecting the dependent variables and interactions between the dependent variables (Box and Behnken, 1960). This design required 46 individual pulping experiments and is described in Appendix 2. Because of operational problems, complete data was not obtained to allow meaningful statistical analysis of the factorial experiment; however, various trends were identified when one variable at a time was examined, which was sufficient for our purpose. These results are described below and the average values (n = 46) are shown in Figures 1 to 3.

3.1.2 Yield of pulped wood

For process economics, pulp yield is the most important factor. One would obviously strive for the highest yield possible without sacrificing other important pulp properties. However, in some processes, such as acid sulphite pulping that aim to remove lignin from the wood fibre, a higher quality pulp is obtained at the expense of lower total pulp yields. Pulp yield was found to be inversely related to HNO_3 concentration and temperature (Figures 1a and 1b, respectively). More lignin and hemicellulose are dissolved into the cooking liquor under these more extreme conditions, thereby causing a lower

residual wood (pulp) fraction. The pulped wood yield ranged from 99 to 68% of original dry wood. Within the ranges studied, phosphoric acid concentration, cooking time and wood:liquor ratio had little effect on pulp yields (Figures 1c - 1d, respectively). Besides opening the fibre structure to allow easier access for the nitric acid, H_3PO_4 does not appear to take part in the reactions during the pulping stage. Cooking time also appears to have a minimal effect on yield in the range studied, indicating that cooking time could be reduced further.

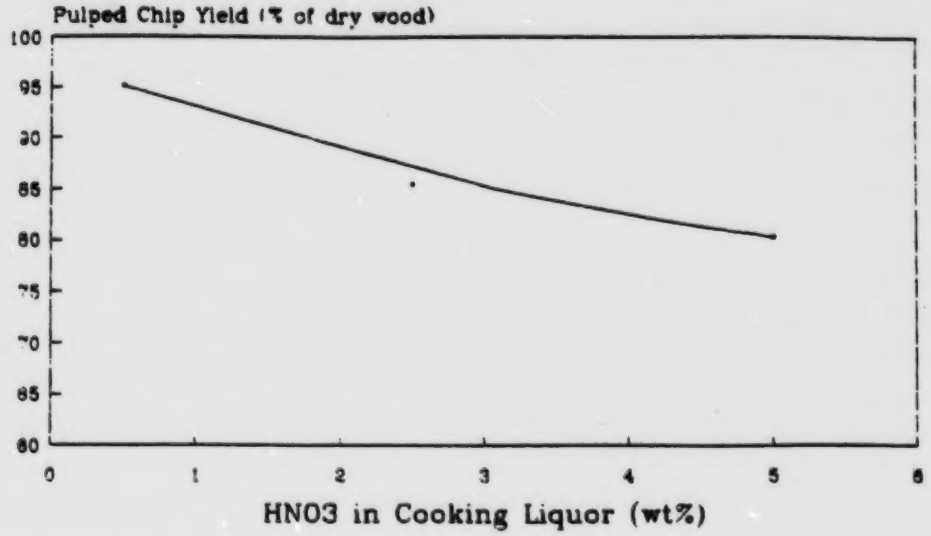
3.1.3 Refining energy

In order to assess the energy needed to refine the pulped chips to pulp, we designed an instrument in which cooked chips were "refined" and the energy consumed was calculated. The energy required to break up the pulped wood chips into individual fibres was found to be inversely related to the nitric acid concentration, cooking temperature and cooking time as shown in Figures 2a, 2b and 2d, respectively. The reduced refining energy reflects the increased amount of lignin removed under the more extreme conditions. As lignin acts as the "glue" keeping the wood fibres together, its removal makes it easier to fiberize the pulped chips. Phosphoric acid concentration and wood:liquor ratio had less apparent effect on refining energy (Figures 2c and 2e, respectively). The apparent refining energy maximum at the wood:liquor ratio of 1:4 (0.25) is actually the result of the interaction between refining energy and HNO_3 concentration. Most of the low (0.5%) HNO_3 concentration cooks tested at 1:4 wood:liquor ratio resulted in significantly higher chip refining energy requirements.

3.1.4 Freeness

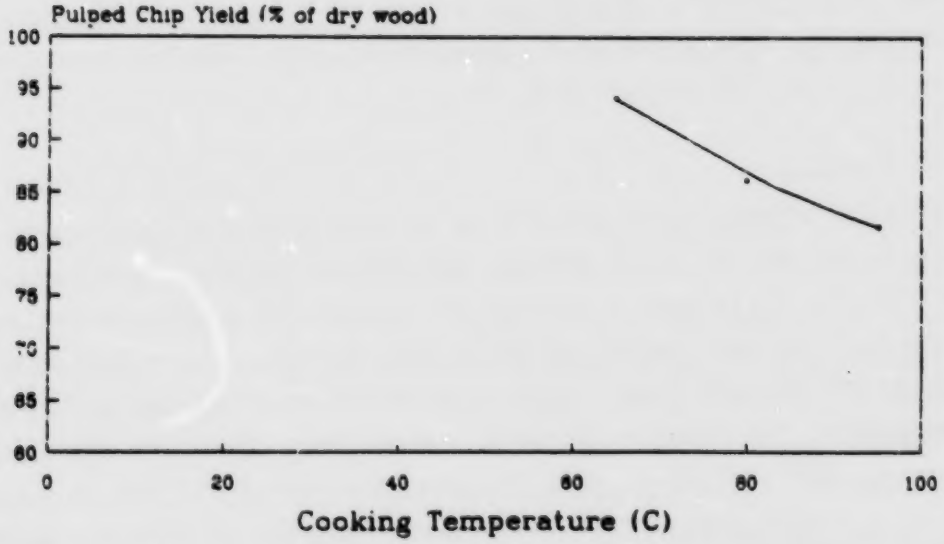
The physical properties of pulp fibres are not only influenced by the amount of lignin and hemicellulose removed, but also by the way the pulped wood chips were fiberized or refined. Refining will affect the surface of the fibre, causing surface fibrillation, which in turn improves bonding and strength in the sheet formed from these fibres. The degree to which the wood is fiberized is measured by the Canadian Standard Freeness Test (CSF). To the papermaker this is an important factor that indicates how fast the fibres will release water when formed into a sheet on the paper machine. It is also customary to

Figure 1a.
Effect of HNO3 on Pulped Chip Yield



Trend only, effect of 1 of 5 variables

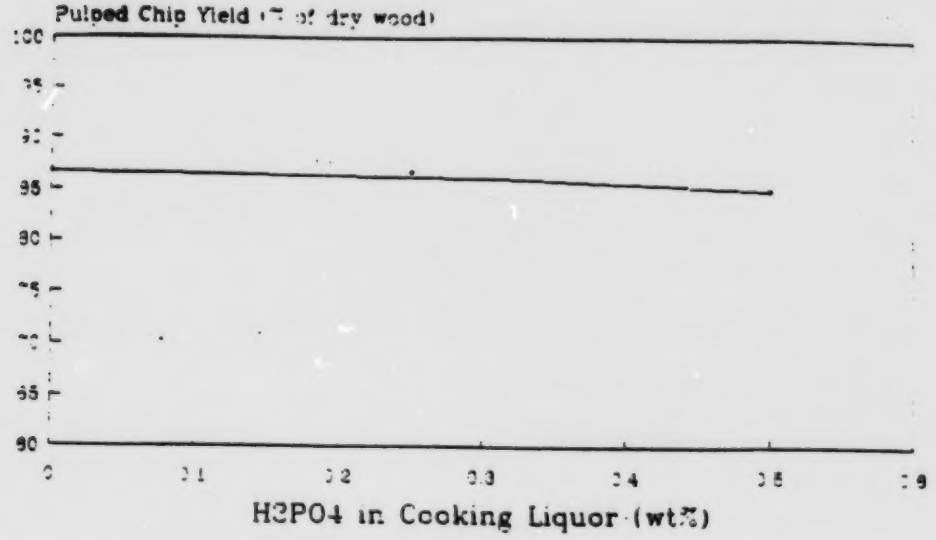
Figure 1b.
Temperature Effect on Pulped Chip Yield



Trend only, effect of 1 of 5 variables

Figure 1c

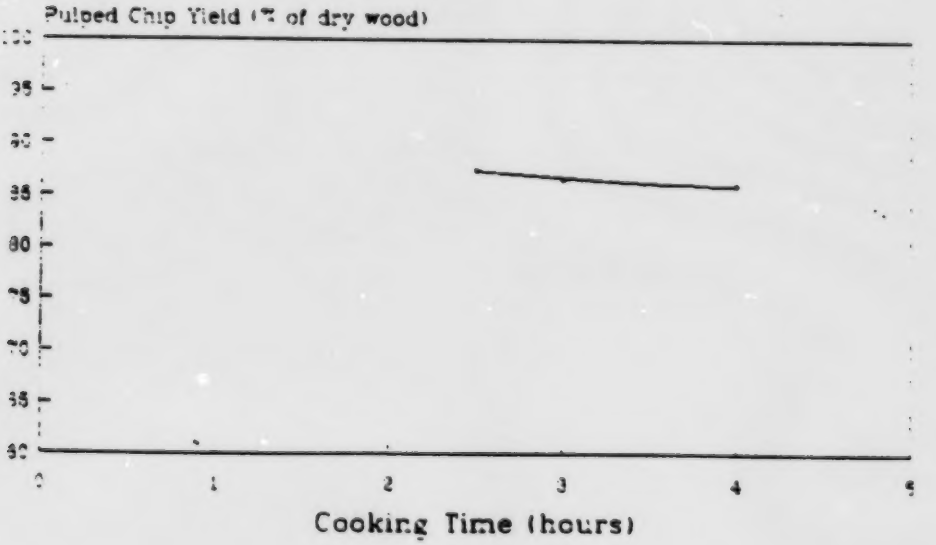
Effect of H3P04 on Pulped Chip Yield



Trend only, effect of 1 of 5 variables

Figure 1d.

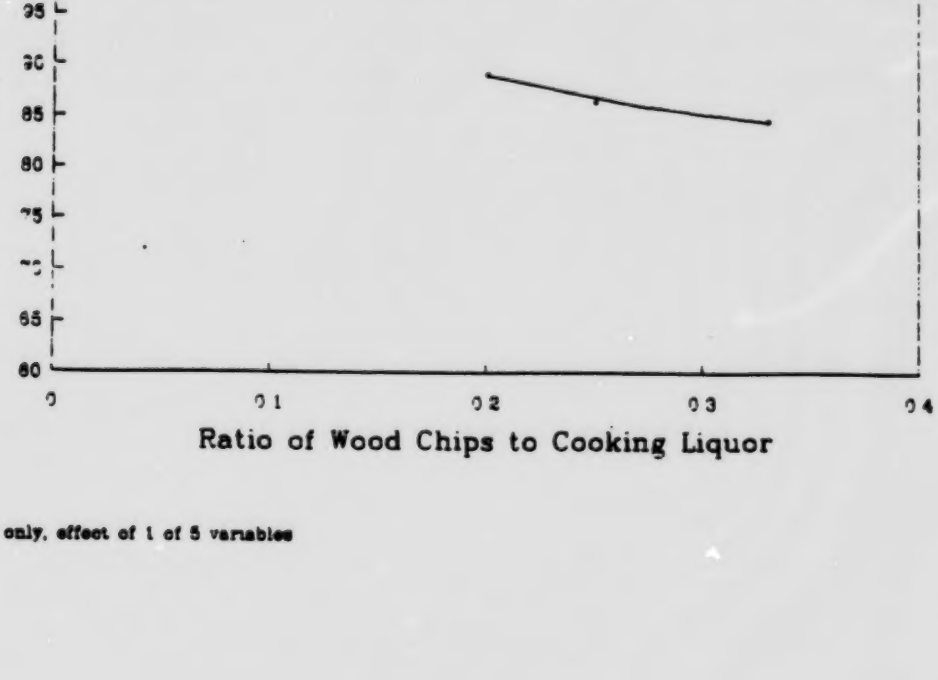
Effect of Cook Time on Pulped Chip Yield



Trend only, effect of 1 of 5 variables

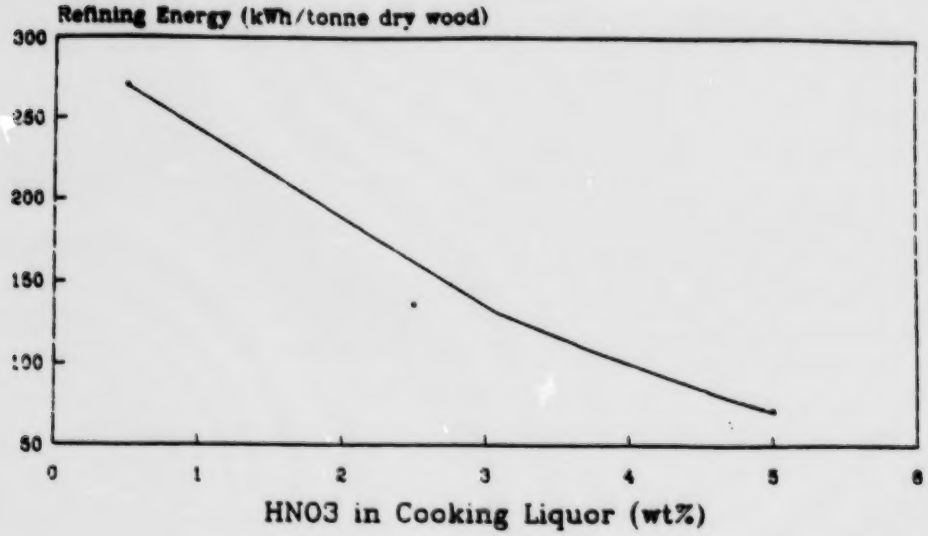
Figure 1e.

Effect of Wood:Liquor Ratio on Yield



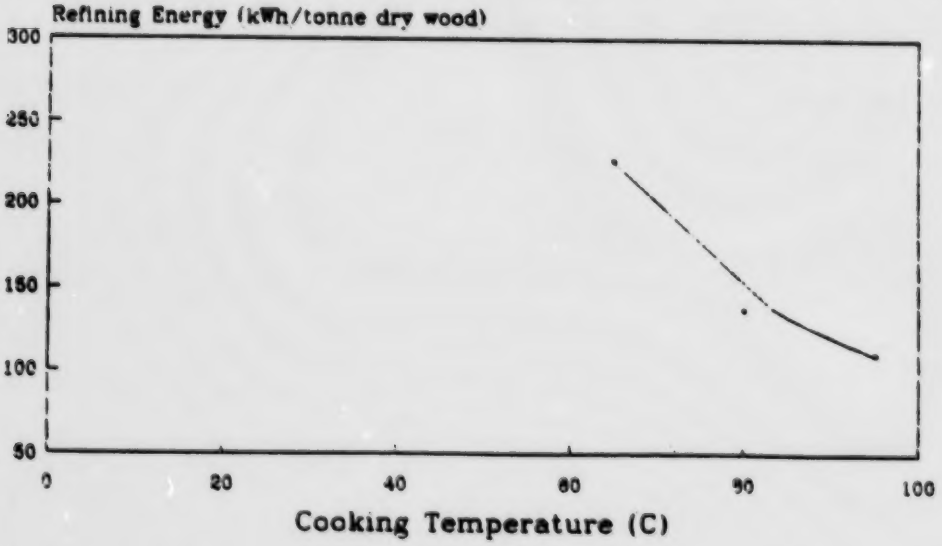
Trend only, effect of 1 of 5 variables

Figure 2a.
Effect of HNO3 on Chip Refining Energy



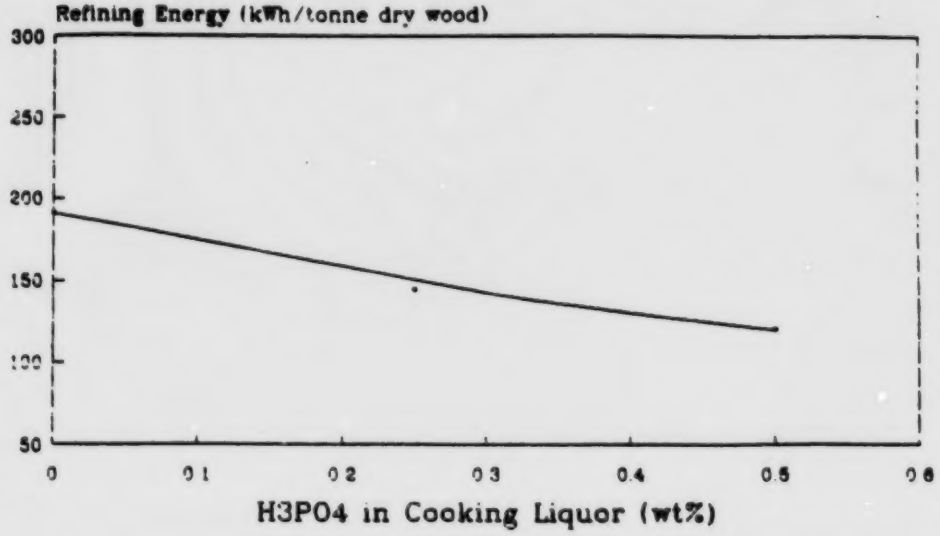
Trend only, effect of 1 of 5 variables

Figure 2b.
Effect of Temperature on Refining Energy



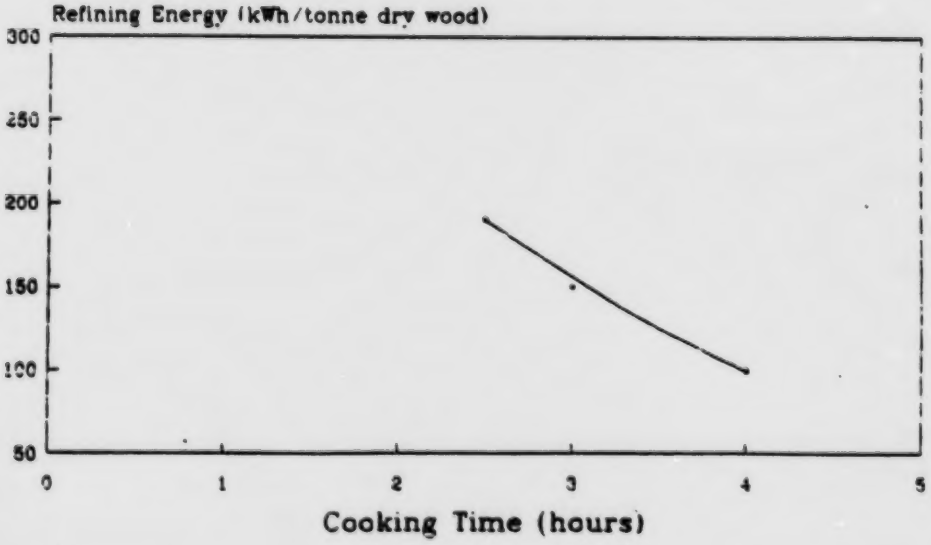
Trend only, effect of 1 of 5 variables

Figure 2c.
Effect of H3P04 on Chip Refining Energy



Trend only, effect of 1 of 5 variables

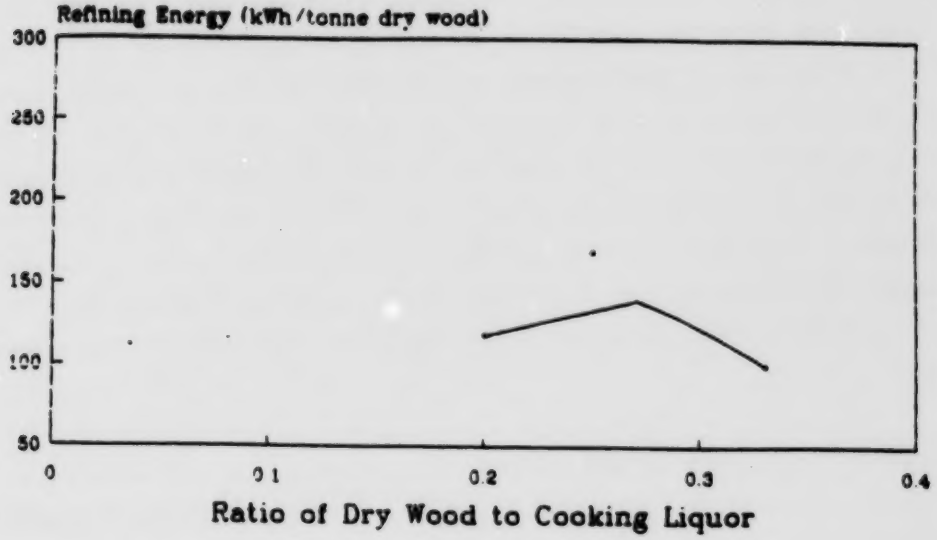
Figure 2d.
Effect of Cook Time on Refining Energy



Trend only, effect of 1 of 5 variables

Figure 2e.

Effect of Wood:Liquor on Refining Energy



Trend only, effect of 1 of 5 variables

express other pulp physical properties at the same CSF value for ease of comparison. Freeness is related to the surface fibrillation and swelling of fibres and is a useful index of the amount of mechanical treatment given to the pulp. The more the wood is fiberized, the lower the CSF value, expressed in millilitres (mL). The freeness values obtained for this experimental series were only approximate because the pulped wood chips were broken up using a Waring blender, which does not effect very good fiberization. Later in the study, Bauer laboratory-scale disk refiners were used, courtesy of both CIP Research, Hawksbury, Ontario; and E.B. Eddy Limited, Hull, Québec. Consequently, these earlier freeness values, ranging from 564 to 850 mL, are much higher than those obtained later using the disk refiner (see Section 3.1.5).

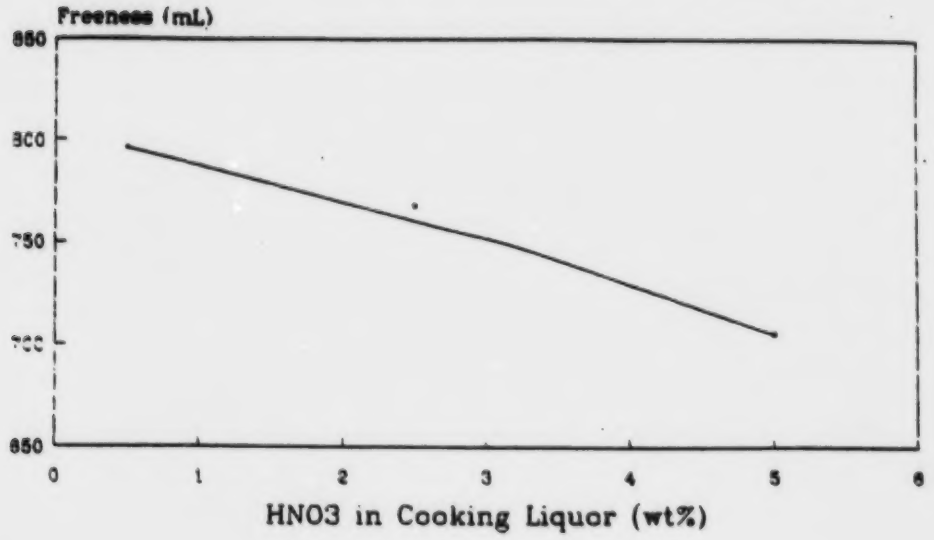
The results in Figures 3a and 3b show that freeness values were inversely related to nitric acid concentration and cooking temperature, respectively. Increasing cooking time from 2.5 to 4 hours showed no significant reduction in freeness (Figure 3d). Similarly, H_3PO_4 concentration and the wood:liquor ratio did not appear to affect freeness (Figures 3c and 3e, respectively).

3.1.5 Physical and optical pulp properties

Optimization of pulp properties was beyond the scope of this project. Consequently, all pulp properties are reported for pulping condition No. 8 (the original base case adopted early in this study). The No. 8 pulping conditions were: 5% HNO_3 and 0.25% H_3PO_4 in the liquor, cooking at 95°C for 3 hours, using an initial dry wood:cooking liquor weight ratio of 1:4. It should be noted that these conditions were selected because they maximized the sugar content of the cooking liquor, which was to be subsequently used for acid hydrolysis of the short fibres and then fermented under acidic pH conditions to ethanol using *Scytalidium acidophilum*. Other cooking conditions may exist that would produce better physical properties of the pulp.

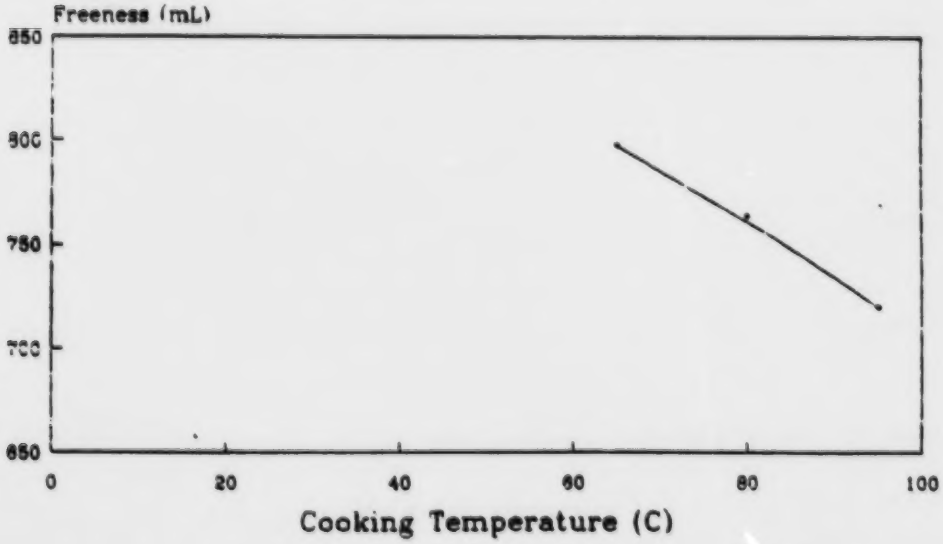
Some of the physical testing results of CRSO pulp produced under No. 8 conditions are shown in Table 2. Also shown are the results of replacing the usual kraft pulp portion of commercial newspaper furnish with CRSO pulp.

Figure 3a.
Effect of HNO3 on Pulp Freeness



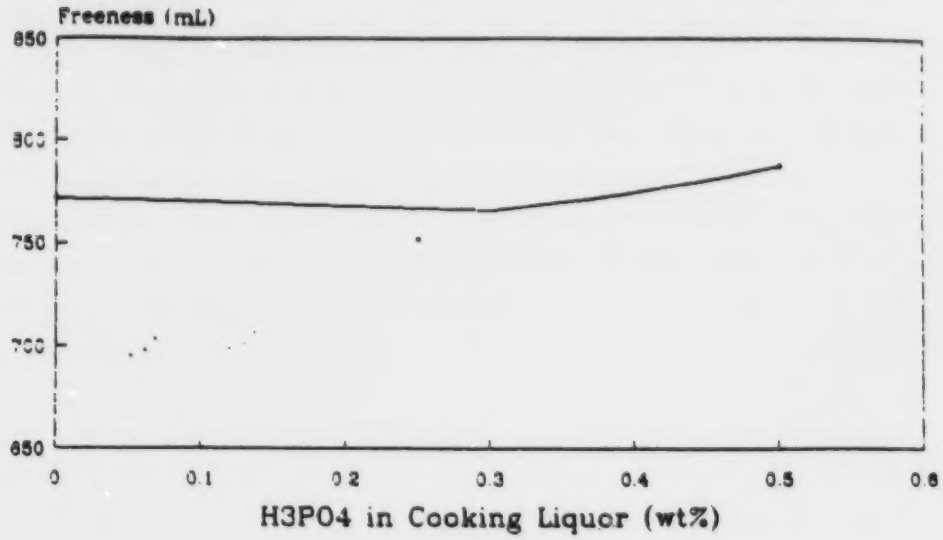
Trend only, effect of 1 of 5 variables

Figure 3b.
Effect of Temperature on Pulp Freeness



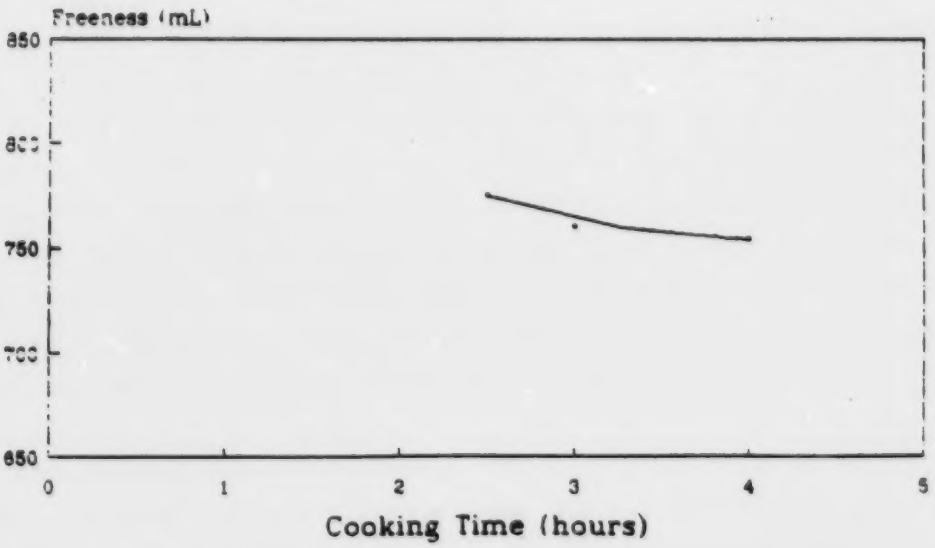
Trend only, effect of 1 of 5 variables

Figure 3c.
Effect of H3PO4 on Pulp Freeness



Trend only, effect of 1 of 5 variables

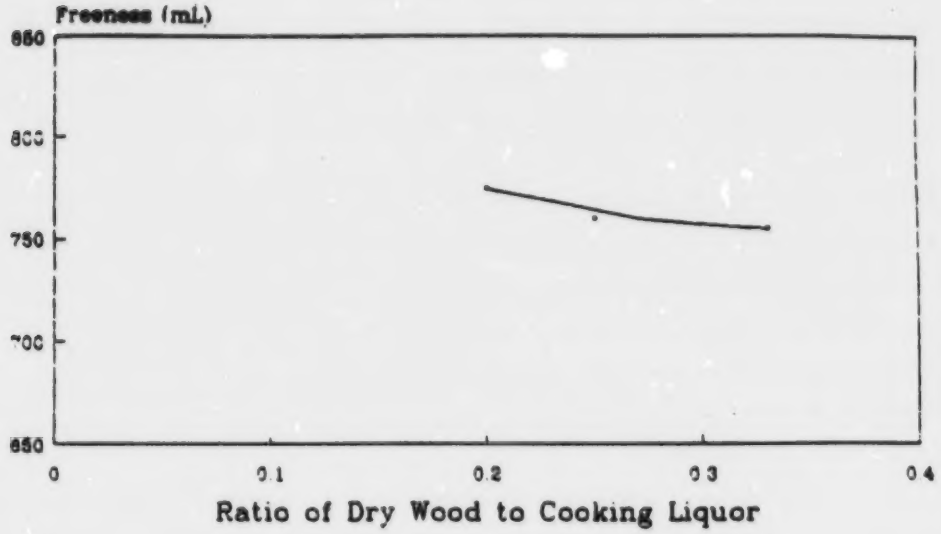
Figure 3d.
Effect of Cooking Time on Pulp Freeness



Trend only, effect of 1 of 5 variables

Figure 3e.

Effect of Wood:Liquor on Pulp Freeness



Trend only, effect of 1 of 5 variables

When making comparisons, one should remember that the comparison is made between a long fibre kraft softwood pulp and the CRSO pulp fibre fraction from birch wood, which is much shorter. The shorter fibres are reflected in the higher **freeness** values for the CRSO pulp. Freeness is described in the previous section. Normally, a good papermaking pulp would have a freeness of 450. Newsprint stock would have a freeness of 100 - 200.

The bursting strength is the hydrostatic pressure, measured in kilopascals (kPa), required to rupture a 7.3 cm^2 circular area of paper. Fibre length and more importantly, the bonding of fibres between themselves, are the main factors contributing to bursting strength. **Bursting index** is the bursting strength required for a given weight (grammage) of sample, expressed here as meganewtons per kilogram (MN/kg). The equivalent unit, kilopascal meters square per gram ($kPa.m^2/g$) is also used in the literature.

The Elmendorf tear test measures the force required to tear four sheets of paper of definite width after the tear has been started. This is a measure of the internal tearing resistance as distinguished from initial or edge tearing resistance. Tear resistance depends almost entirely on fibre length, and its measurement is often used to indicate good or bad operating strength on web fed printing presses. The units used for **tear index** are millinewton meters square per gram ($mN.m^2/g$).

Tensile strength tests indicate the resistance of paper to breaking when subjected to a longitudinal strain, measured in kilonewtons per metre (kN/m). The **percent stretch** at the time of rupture is also recorded during this test. The **tensile energy adsorption (TEA)** of the sample is the work done when a sample is stressed to rupture in tension under prescribed conditions as measured by the integral of the tensile stress over the range of tensile strain from zero to maximum strain. TEA is expressed as energy per unit area of sample, specifically, Joules per meter square (J/m^2). **Tensile breaking length**, expressed in kilometres (km), is the calculated limit length of a strip of paper beyond which it would break under its own weight if suspended by one end.

Tensile strength is a measure of the bonding strength of the sheet and generally correlates well with bursting strength, but not with tear. Tensile strength of printing papers is used to indicate the potential resistance to breaking during printing and other converting operations. From the data in **Table 2**, it is obvious that the CRSO pulps all had good tensile strength properties compared to newsprint furnish.

Optical tests of the pulp include brightness and opacity. **Brightness** is a measure of the amount of light reflected by the surface of the sample when it is illuminated with blue light (approx. 457 nm wavelength) using a diffuse geometry type instrument. ISO Brightness is reported as a percentage of a perfectly reflecting and diffusing surface (the absolute standard). Brightness is a measure of the effective bleaching or whiteness of the pulp.

Opacity in a sheet of paper indicates how impervious it is to the passage of light. Opacity is expressed as a percentage of the reflectance of light when the paper is placed over a black cavity (background) compared to when a white backing of 89±0.5% reflectance is used. High opacity is important for pulps used in printing papers and envelopes. The use of ammonia bleached CRSO pulp in newsprint furnish produced adequate brightness and superior opacity compared to the kraft-based newsprint furnish.

Overall, the results shown in **Table 2** indicate that the tear index of paper made with 100% CRSO pulp may be too low for use as newsprint furnish, but replacing kraft pulp in newsprint furnish with an optimized birch CRSO pulp is definitely feasible and that there should be a ready market for this pulp.

Table 2. Physical Properties of CRSO Base Case Pulp [1]

	Pulp Composition						
	100% CRSO				12.8% CRSO in newsprint[2]		12.8% kraft in newsprint
Sample Number:	1	2	3	4	5	6	7
Pulp Treatments							
Primary Cooking Time (h)	3	3	3	3	3	3	-
Secondary Cooking Time (min)	0	20	20	30	0	30	-
Bleaching Agent	None	None	NaOH	NH_4OH	None	NH_4OH	-
Physical Properties							
Freeness (mL)	291	313	306	NA	82	101	130
Bulk Index (cm^3/g)	1.8	1.4	1.3	1.2	2.3	2.3	2.4
Burst Index (MN/kg)	3.0	1.8	1.9	2.2	2.0	1.6	1.8
Tear Index ($mN.m^2/g$)	4.5	1.9	2.2	2.5	6.0	4.9	7.6
Tensile Strength (kN/m)	3.5	3.4	2.8	3.4	2.5	2.3	2.2
Tensile Energy Adsorption (J/m^2)	42.9	32.1	28.2	27.4	31.4	24.6	26.8
Stretch (%)	1.9	1.6	1.6	1.4	2.0	1.8	1.9
Tensile Breaking Length (km)	5.7	5.4	4.5	5.6	4.1	3.8	3.7
Optical Properties							
Brightness (%)	24.6	38.7	69.6	57.5	51.1	55.5	57.9
Opacity (%)	85.5	83.2	82.2	77.3	89.6	96.3	91.0

(1) CRSO pulp from No. 8 cooking conditions, fiberized on Bauer disk refiner, Bauer-McNett classification R28+R48

(2) Standard newsprint furnish minus kraft pulp component: 20.2% chemi-mechanical pulp, 23% refiner groundwood, and 44% stone groundwood.

3.2 Fibre Separation

Fibre length is a fundamental property of pulp as it affects many of its strength parameters like burst, tear, and tensile strength. The fibres can be characterized by fibre length using a Bauer-McNett classifier, which separates a pulp sample by a cascade series of four screens of 28, 48, 100 and 200 mesh. The long fibre fraction (80 mesh, for pulp use) was separated from the short fibre (fines) fraction, after refining of the pulped wood chips. This was done by suspending the whole pulp in water and running it over a screening device that allowed the fines to pass through, while retaining the pulp fibre fraction. A 40X50 wires per inch polyester mesh, similar to that used in a paper machine, was found to work satisfactorily. On a commercial scale, one would separate the fibre fractions with rotating screens as is done in the paper mills. For the pulp from a No. 8 cook of white birch chips, screening in this manner separated the fractions to yield on average 70% long fibre pulp and 30% short fibre fines.

3.3 Bleaching

The pulp produced can be used in the unbleached state or it can be bleached to a higher degree of whiteness by extraction with sodium hydroxide (caustic soda, NaOH), which removes the nitrated lignin. Extraction with 5 to 10% NaOH can be carried out directly on the whole pulp, before separation, or on the long fibre fraction only. We found that 5 to 10% ammonium hydroxide (NH_4OH) also works well for bleaching. Ammonia bleaching has the advantage over NaOH because it costs less and once used, it can be easily disposed of by addition to spent cooking acid to neutralize and produce an ammonium nitrate fertilizer by-product.

To achieve higher degrees of brightness, the pulp was treated once more in 1 to 5% HNO_3 at 95°C for 20 to 30 minutes, followed by extraction with 5 to 10% sodium or ammonium hydroxide (see Table 2, sample 1 versus 3 and 4). Significant increases in brightness from 24.6% for unbleached pulp to 69.6% (NaOH) and 57.5% (NH_4OH) were observed following only one extraction. This is a significant finding in light of the growing concern over the environmental hazards of chlorine bleaching of kraft pulp.

3.4 Simultaneous enzymatic hydrolysis and fermentation (SHF)

Our original plan was to use a secondary HNO_3 hydrolysis of the cellulose in the short fibre fraction to produce glucose for fermentation. Because of the apparent resistance of the fines to acid hydrolysis, discussed in Section 3.1.1, enzymatic hydrolysis was used. To eliminate glucose inhibition of the cellobiase (β-glucosidase) activity, a simultaneous hydrolysis and fermentation (SHF) procedure was adopted. Because the enzymes require a pH around 4.5 for maximum activity, the use of the acidophilic yeast, *Scytalidium acidophilum*, originally proposed for fermentation of acid hydrolysates, was abandoned in favour of commercial bakers' yeast, *Saccharomyces cerevisiae*. A high initial yeast cell density (6 wt% on dry wood fines) was used to ensure more rapid removal of glucose by fermentation than glucose production by enzymatic hydrolysis of cellulose. This ensured that glucose inhibition of enzymatic hydrolysis did not occur. The enzymatic hydrolysis of cellulose is normally the rate limiting step in the SHF process.

The enzymes used in SHF were **cellulase** (EC 3.2.1.91, EC 3.2.1.74, EC 3.2.1.4) (Novo Celluclast 1.5L or ASCAF CSB 04-06-L10), **cellobiase or β-glucosidase** (EC 3.2.1.21) (Novozyme 188), and in some initial experiments a **protease** (Novo Neutrase). The liquid enzyme preparations were added to obtain the following enzyme loadings expressed in mL/100g dry wood fines: 4.0 cellulase, 0.8 cellobiase and 0.01 protease.

Protease was added in initial SHF studies to possibly reduce the size of cellulase molecules so as to allow them better penetration into openings in the crystalline cellulose of the fines. Molecular weight determinations of the treated and untreated cellulase to confirm the size reduction by protease treatment were not attempted. While the rate of enzymatic cellulose hydrolysis alone (i.e., in the absence of yeast) was found to increase when protease was added (see Appendix 1, p 23), later studies showed no significant difference in SHF results when protease was omitted, consequently its use in SHF was discontinued.

Standard cellulase activity assays such as the filter paper unit (FPU) assay were not run to determine the initial activity of the enzyme stock solutions

as received from the suppliers. Novo Nordisk Bioindustrials, Inc. (Danbury, CT) product literature states that stock enzyme activity should be stable for up to 3 months at 25°C and "at lower temperatures (5 - 10°C), the shelf life is considerably increased". All stock enzymes were stored at around 7°C. Since the SHF experiments were run over a period exceeding one year using the same enzyme stock solutions, the enzyme activity present in later SHF experiments may have been less than when used initially, despite using the same volume of enzyme stock. Enzyme activity assays were run at the end of this study to obtain a comparative estimate of enzyme loading used in the SHF experiments. The results summarized in Table 3 are probably lower than actually used in initial SHF experiments. Reported cellulase activities used in SHF, range from 7 to 30 FPU/g substrate, depending on the source and pretreatment of the cellulose (Hogan *et al.* 1990, Wright *et al.* 1988). If we estimate 55% cellulose in the pulped birch fines, the minimum cellulase activities used in our SHF studies was between 25 and 40 FPU/g cellulose. These figures indicate that excess cellulase may have been used in our SHF studies and it may be possible to reduce enzyme levels without significantly reducing SHF rate.

Table 3. Enzyme Activities Used in SHF Experiments

Enzyme	Supplier	FPU/mL[1]	CBU/mL[2]	SHF Loading (Units/g dry wood)
Celluclast 1.5L	Novo Nordisk	345	-	14 FPU/g
Cellulase	ASCAF[3]	483	-	20 FPU/g
Novozyme 188	Novo Nordisk	-	5000	40 CBU/g

(1) Filter Paper Units.
(2) Cellobiase Units.
(3) Association pour le développement des carburants par la fermentation, Soustons, France.

The resultant activity of cellulase on cellulose forms primarily cellobiose, a β(1-4) disaccharide of glucose. The cellulase preparations contain a certain

level of cellobiase activity, which hydrolyses each cellobiose molecule to form two glucose molecules. The presence of excess cellobiose during enzymatic hydrolysis of cellulose can cause inhibition of cellulase activity. Excess cellobiase activity is required to minimize this inhibition.

To determine if sufficient cellobiase was present in the ASCAF CSB 0406 cellulase preparation, a SHF of 15 wt% pulped birch fines was run with and without the addition of Novozyme 188 cellobiase. The results shown in Figure 4 clearly indicate the enhanced rate of ethanol production by SHF when cellobiase was added. The efficiency, with respect to the theoretical cellulose hydrolysed and ethanol yield for the SHF with added cellobiase, is shown in Figure 5.

Theoretical values were determined based on 0.51 g ethanol/g glucose fermented and an estimated cellulose content of the fines of 55 wt%. Actual analysis of the fines, done by Bio-Hol Developments of Toronto, were 50.1% cellulose, 14.4% hemicellulose and 21.3% lignin, representing only 85.8% of the total dry weight. The use of 50.1% in these theoretical calculations of cellulose hydrolysis, based on ethanol produced, resulted in several values in excess of 100%. Consequently, 55% cellulose content was used as a more conservative value.

Figure 5 shows that at an initial substrate concentration of 15 wt%, SHF over 120 hours allowed 90% cellulose hydrolysis and 73% of the theoretical ethanol yield. These results demonstrate that the CRSO nitric acid pulping process renders the cellulose in the pulp fines very susceptible to enzymatic hydrolysis.

The effect of chip cooking time on SHF was studied using initial substrate concentrations of 21 wt% fines. Three cooking times, 0.5, 1, and 3 hours, were used to prepare the pulp from which the fines were recovered. SHF was carried out using Novo Celluclast and Novozyme 188 cellobiase supplementation. The results in Figure 6 showed a slight improvement in the 1 hour cook over the other two. Even if the improvement is negligible, this is a significant finding because it indicates the potential for reducing chip cooking time from

Figure 4.

SHF of 15 wt% Pulped Birch Fines

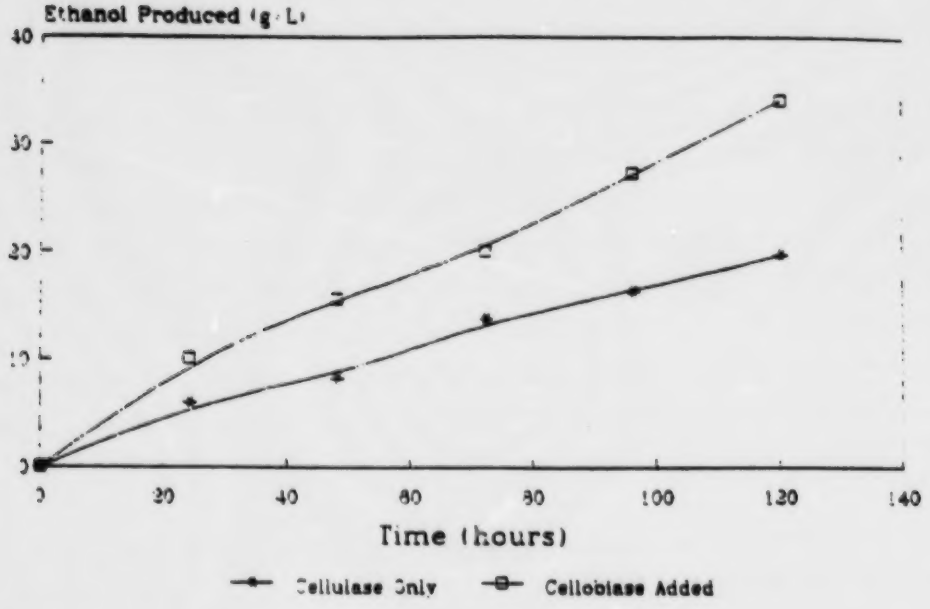
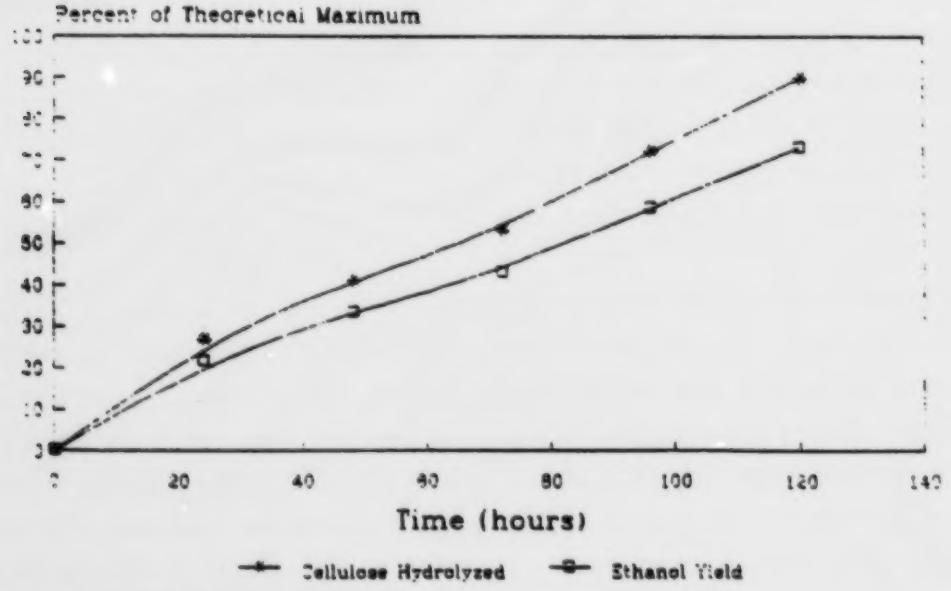


Figure 5.

Efficiency of SHF of 15 wt% Birch Fines



SHF USING CELLULASE PLUS CELLOBIASE

the 3 hours used in the base case to 1 hour or less, without reducing enzymatic hydrolysis of the fines. However, cooking time will be determined by the required quality of pulp produced, because it is the highest value product of the CRSO process.

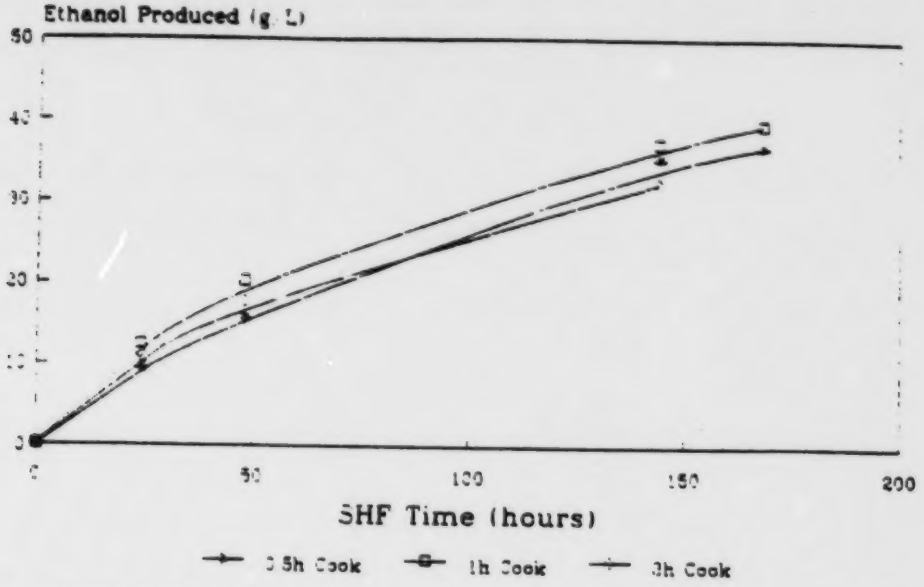
Figure 7 shows the efficiency of the 21 wt% SHF for the 1 h cooking time data. These results show that SHF at 21% appeared less efficient than at 15% under the standard conditions (see Figure 5), although a lower cellulase loading may have been used. The reaction was not complete after 168 hours. The higher solids content in the static (unmixed) fermentation tubes probably contributed to the reduced SHF efficiency and longer reaction time required for 21% fines. Higher ethanol concentration, approaching 40 g/L may also have had some inhibiting effect on the cellulase complex as reported by others (Ooshima *et al.* 1985).

SHF of 10 wt% fines was tested to eliminate the higher ethanol and solids concentration effects. Figure 8 shows that the SHF using Celluclast and Novozyme 188 cellobiase was completed by 48 hours. Figure 9 shows that SHF at 10% substrate concentration gave the same high efficiency as that observed for 15% (see Figure 5). These results are typical of over ten SHF runs completed using 10 wt% fines.

The SHF productivities over the first 24 and 48 hours and at the time of maximum ethanol production for each of the substrate concentrations tested are shown in Table 4. Under the standard SHF conditions used, the use of higher substrate concentrations reduces productivity because of the longer reaction times required, but higher final ethanol concentrations are produced.

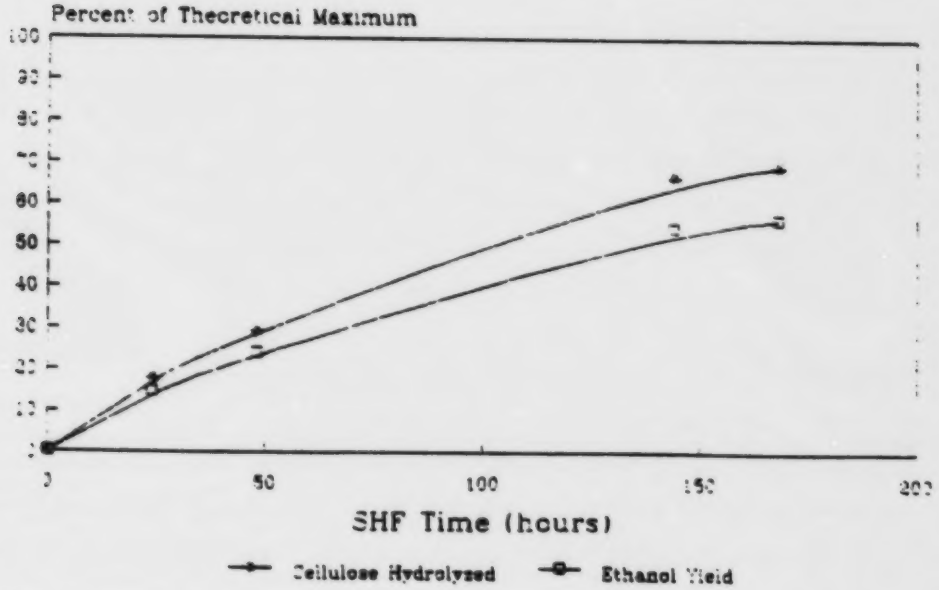
While higher ethanol concentration in the final beer is desirable for easier product recovery, the longer fermenter residence time required at the higher substrate concentrations would lead to higher capital costs in fermenter capacity. Several approaches to reducing the SHF time for high substrate concentrations remain to be tested. These include proper mixing of substrate, enzymes and yeast; the use of higher or optimal enzyme loadings; the use of thermotolerant yeasts to allow SHF at temperatures closer to the optimum for

Figure 6.
Cook Time Effect on SHF of 21 wt% Fines



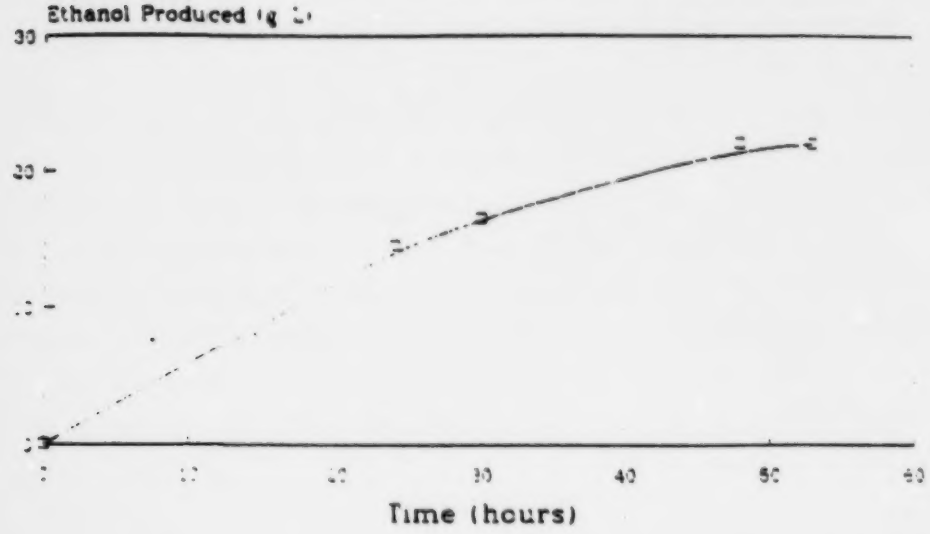
SHF USING CELLULASE PLUS CELLOBIASE

Figure 7.
Efficiency of SHF of 21 wt% Birch Fines



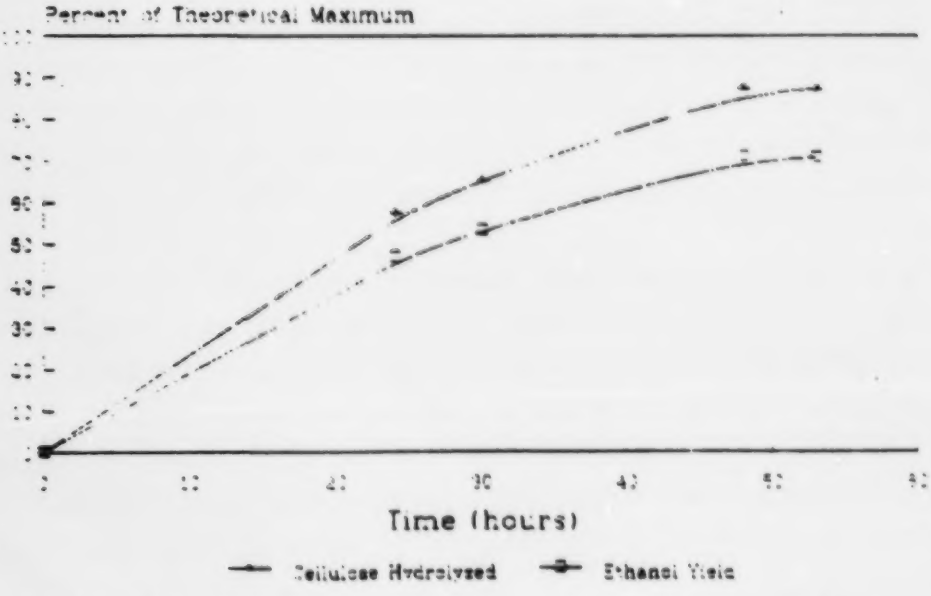
DATA FOR 1h COOKING TIME

Figure 8.
SHF of 10 wt% Pulped Birch Fines



SHF USING CELLULASE PLUS CELLOBIASE

Figure 9.
Efficiency of SHF of 10 wt% Birch Fines



SHF USING CELLULASE PLUS CELLOBIASE

enzyme activity (i.e., 45°C instead of 30°C); the use of pH control; and the use of fed-batch SHF to avoid the problems associated with mixing initial high solids. Several of the above suggested improvements could also lead to reduced enzyme requirements resulting in improved process economics.

Table 4. Simultaneous Hydrolysis and Fermentation of Pulped Birch Fines[1]

Fines (wt%)	SHF Time (h)	Ethanol Produced (g/L)	% Theoretical Cellulose Used[2]	Productivity (g Ethanol/L/h)
10	24	14.5	57.3	0.60
	48	22.1	87.3	0.46
15	24	10.1	26.8	0.42
	48	15.5	40.9	0.32
	120	34.1	89.7	0.28
21	24	12.3	23.2	0.51
	48	20.1	37.8	0.42
	168	39.5	74.2	0.23

(1) All fines were <R48 fractions of chips pulped under base case (No. 8) conditions. SHF used cellulase plus cellobiase.
(2) Based on ethanol produced and estimated 55 wt% cellulose initially present in unbleached birch fines.

3.5 By-product utilization

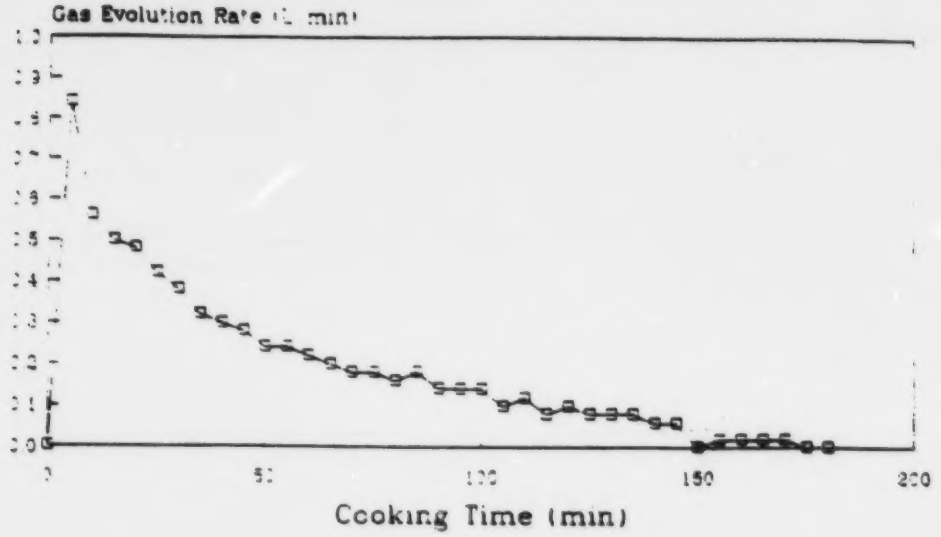
3.5.1 Reaction gases

During the pulping reaction there are gases produced. A series of birch chip cooks were run to obtain data on the volume of gas produced. The process gas evolution rate and cumulative volume produced are shown in Figures 10 and 11, respectively. Gas production rate peaked within the first 10 minutes of the reaction at 95°C. Total gas evolution after a 3 h cook reached 35L for 500 g of dry wood chips. This translates into 70 m^3/tonne dry wood.

The composition of the gas is not completely known. The components identified are shown in Table 5. The reaction gases could be ignited, but proper air injection was not available to sustain combustion by the heat generated. A properly designed gas incinerator and gas scrubbing system can be designed to treat the reaction gases. Theoretically, after ignition the HCN and NO can be

Figure 10

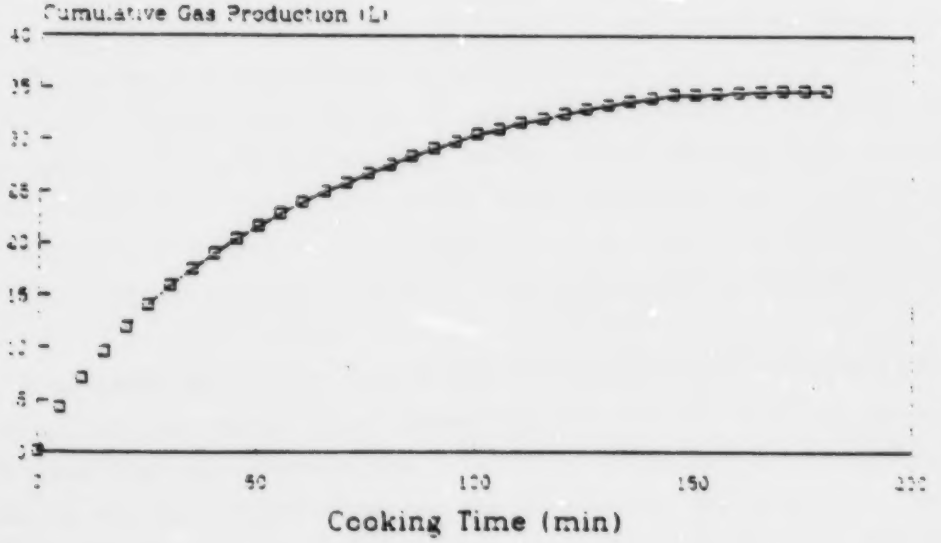
Gas Evolution Rate During Pulping at 95C



PULPING OF 500 G DRY WT BIRCH CHIPS

Figure 11.

Gas Production During Pulping at 95C



PULPING OF 500 G DRY WT BIRCH CHIPS

completely oxidized to NO_2, which can then be absorbed in water to form HNO_3 for reuse in the cooking liquor. Demonstration tests of this proposed acid recovery process remain to be carried out.

Table 5. Partial Composition of CRSO Process Reaction Gas

Component	Cooking Temperature (°C)	Concentration (ppm)
Hydrogen cyanide (HCN)	25	0
	75	8
	87	>370[1]
	95 (after 1 h)	100
Nitric oxide (NO)	95	>1000
Nitrogen dioxide (NO_2)	85	>30
Nitrous oxide (N_2O)	95	1500
Acetic acid (CH_3COOH)	95	400
Formic acid (CHOOH)	93	600

(1) ">" indicates that amount exceeded the calibration limit concentration used.

3.5.1.1 Cyanide removal

Because of the acute toxicity of cyanide, additional studies were carried out to determine ways of minimizing HCN in the reaction gas, used cooking liquor and wash water. Our initial cooking apparatus used a vertical condenser that allowed condensate to reflux, returning to the cooking vessel. The effect of using a horizontal condenser to remove the HCN condensate from the reaction gas in order to reduce HCN levels in the cooking liquor and wood pulp was tested.

The results shown in **Table 6** confirm that the condensate collected by the horizontal condenser contained a high HCN concentration. After adjusting the condensate pH to 10 with NaOH to facilitate HCN oxidation by hydrogen peroxide, the results confirmed that with sufficient H_2O_2 present, total HCN oxidation could be achieved. In theory, at high pH, an equimolar amount of H_2O_2 and cyanide is needed to oxidize the cyanide to CO_2 and N_2. From these results, 1000 ppm H_2O_2 was insufficient, but approximately 5000 ppm would probably suffice to oxidize the 4080 ppm present.

Removal of the cyanide in the reaction gas would be preferably carried out by gas incineration as mentioned above, because of the cost that hydrogen peroxide treatment would add to the process.

Table 6. Cyanide Analysis of Cooking Liquor and Condensate[1]

Sample Type	Condenser Orientation	H_2O_2 Added (ppm)	pH	Cyanide (ppm)
Condensate	Horizontal	0	1.4	3830
"	"	0	10.1	4080
"	"	1000	10.1	2160
"	"	10000	10.1	<0.1
Cooking Liquor	"	0	0.7	587
1st Wash Water	"	0	1.3	93
Cooking Liquor	Vertical	0	0.7	649
1st Wash Water	"	0	1.3	89

1 - From 500g birch chips cooked for 3h at 95°C. Total condensate recovered was 65.0 mL.

The effect of condenser orientation on reducing HCN in the cooking liquor was not as great as was hoped, but the horizontal condenser allowed an apparent decrease of 10%. Significance of this reduction has not yet been confirmed.

Similarly, no significant reduction in the residual HCN in the first wash waters of the pulp was apparent. The use of a more efficient condenser may trap a higher concentration of the produced cyanide. Our condenser used cold tap water, which could not keep the exhaust temperature below 20°C because these studies were done during the summer months. HCN boiling point is 26°C.

Another study was done to determine the cyanide levels in the beer following SHF, before distillation. The effect of H_2O_2 addition to the fermentation mixture before separation of the beer was also examined. Both NaOH and NH_4OH were tested for use in adjusting the pH to 10 prior to peroxide treatment. The results shown in **Table 7** revealed that less than 2 ppm cyanide was present in the beer. The addition of 100 ppm H_2O_2 was effective in reducing this level to <1 ppm, but higher H_2O_2 concentrations appeared to increase the cyanide level. It may be that the reaction of the H_2O_2 with the lignin, as evidenced by the evolution of gas when 1000 and 10000 ppm H_2O_2 was added, released some residual cyanide from the lignin. However, these apparent increases may not be significant. Because of the low level of cyanide present, it appears that H_2O_2 treatment of the fermentation residues (yeast and lignin solids) will not be necessary to render them safe for use as animal feed.

Table 7. Residual Cyanide Analysis and H_2O_2 Oxidation in SHF Beer

pH adjusted to 10 using	H_2O_2 added ppm	Cyanide ppm
NaOH	0	1.71
	100	0.79
	1000	2.39
	10000	3.32
NH_4OH	0	1.04
	1000	1.33

3.5.2 Sugars in used cooking liquor and pulp wash water

The used cooking liquor contains glucose, xylose, mannose, galactose, and acetic acid, formic acid and lignonitrates in various amounts, depending on the cooking conditions used. A partial analysis of the used cooking liquor from a base case cook found 3.5% xylose, 0.5% glucose, 1.0% acetic acid, 0.1% formic acid and 1.4% lignonitrate present. Preliminary used acid fermentations at pH 1.3 showed that utilization of xylose, the major sugar component, was negligible and glucose fermentation was very slow when the mycelium form of *Scytalidium acidophilum* was used. The sugars could be partially fermented to ethanol at pH 4.5 by the yeast form of *Scyt. acidophilum.*

We attempted to enhance the xylose fermentation ability of *Scyt. acidophilum* through a strain improvement program carried out by Dr. Duan Zhi-Zhong, a visiting Chinese scientist. Several mutants were obtained and a xylose fermentability selection method was devised for screening these mutants. Unfortunately, these studies remain to be completed before improved xylose fermentation by *Scyt. acidophilum* becomes a reality. The preliminary fermentation work by Dr. Duan Zhi-Zhong is reported in **Appendices 3 and 4.**

Another approach to treating the used acid would be to neutralize it with used ammonium hydroxide from the pulp bleaching step. This would yield a solution containing ammonium nitrate, which could be used as a liquid fertilizer. Another possibility would be to concentrate and dry the neutralized acid for use as a solid fertilizer or soil conditioner. These options have not yet been tested.

The pulp wash water, refiner water and water used in the separation of the long and short fibre fractions also contain various amounts of sugars, primarily xylose, and volatile organic acids that contribute to the biochemical oxygen demand (BOD) of this waste stream. In order to reduce BOD, these waste waters were collected and treated in a 10 cm x 122 cm up-flow column reactor containing mycelium of *Scytalidium acidophilum.* The results after one and two passes of the wash water through the column reactor are shown in **Table 8.** After one pass, xylose concentration was reduced by 39%.

After the second pass, xylose concentration was reduced by 48%. Organic acid concentrations were also reduced by this treatment. With sufficient hydraulic residence time and an adequate bed of active mycelium, nearly all of the sugars and organic acids could be removed. In addition, it was noticed that a certain amount of the yellow colour in the waste water was removed as well. The up-flow reactors could be more adequately aerated in order to produce more biomass to be sold for animal feed. Further research is necessary to define the aeration requirements and residence time required for maximum BOD removal. The suitability of *Scytalidium acidophilum* as chicken feed has been demonstrated by studies at Agriculture Canada (Sibbald and Ivarson, unpublished data cited by Miller *et al.*, 1985).

Table 8. Treatment of Wastewater by *Scytalidium acidophilum* Column Reactor

Number of passes through column	Xylose (g/L)	Percent Reduction	Formic acid (g/L)	Percent Reduction	Acetic acid (g/L)	Percent Reduction
0	11.4	-	1.2	-	4.4	-
1	7.0	39	0.31	74	2.9	34
2	5.9	48	0.25	79	2.4	46

3.5.3 Residual solids remaining after distillation

After distillation of the ethanol from the fermented hydrolysed fines, the residual solids containing heat-inactivated yeast, pentoses and lignin may be concentrated by a suitable de-watering process and then sold as an animal feed supplement or a fertilizer-soil conditioner after composting. Tests of these treatments for the disposal of these residual solids were not attempted during this study.

3.6 CRSO pilot plant studies

Eight pilot plant cooks were completed using commercial size white birch chips. The pulping conditions used in each run are shown in **Table 9.** The first five cooks used the base case conditions, varying only the cooking time. In general, pulp yield was significantly lower than with the bench scale cooks (see **Figures 1a-e**). This reflects the more rapid attainment of cooking temperature that was possible in the steam-heated pilot plant reactor. This presumably led to more lignin and hemicellulose removal from the wood chips during the same cooking times. After refining, the long fibre pulp fraction (>R 80) was about 65 to 75% of the total pulp. Reducing the cooking time from 3 to 0.5 hours reduced the long fibre fraction by about 10% (Cook No. 5). A similar reduced yield of long fibre pulp was obtained after refining pulp from Cook No. 7, which used both reduced nitric acid concentration and cooking time. Total pulp yield was much higher (94%), indicating a lesser degree of lignin and hemicellulose removal during pulping.

As expected, the higher yield cooks, Nos. 6, 7 and 8 all produced inferior quality pulps as shown in **Table 10.** The lowest yield pulp, produced by Cook No. 3, was compared to the whole pulp, before and after separation of the fines from the pulp, as shown in **Table 11.** The effect of not separating the fines from the >R 80 fraction of the pulp was a slight lowering of the pulp strength properties, although the freeness of the whole pulp was significantly less, making direct comparisons difficult.

The effect of bleaching and further fractionation of the longer fibre pulp (>R 48) from the >R 80 pulp fraction that was normally separated by the pilot plant vibrating screen apparatus, is also shown in **Table 11.** The longer fibre fraction produced a slightly reduced pulp quality in all aspects. Bleaching was very effective with both pulp fractions. These results indicate that the >R 80 fraction, approximately 70% of the total wood fibre, was the most suitable for sale as pulp.

Table 9. Pulp Production Parameters Used in Pilot Plant Cooks and Pulp Yields after Refining

Cook Number:	1	2	3	4	5	6	7	8
Vacuum used for 1h (mm Hg)	508	533	533	381	508	381	279	330
Nitric acid (wt% in liquor)	5.0	5.0	5.0	5.0	5.0	1.8	2.5	2.5
Phosphoric acid (wt%)	0.25	0.25	0.25	0.25	0.25	0.16	0.25	0.25
Cooking time (h)	2.5	3.0	3.0	3.0	0.5	3.0	0.5	3.0
Pulp yield (%)	NA	63	58	62	72	77	94	83
Disk refiner gap ($\times 10^{-3}$ in.)	12	10	6	6	6	6	6	3
Refiner passes	4	3	3	2	4	5	5	4
Bauer-McNett Classification (wt%)								
R 28	18.3	21.1	8.9	12.4	13.2	33.8	31.2	34.1
R 48	55.6	54.6	85.5	85.3	52.5	42.7	31.2	36.6
R 100	12.3	5.7	9.5	9.4	11.0	8.4	8.4	7.3
R 200	6.5	2.0	5.6	5.1	7.1	5.9	5.2	3.4
R <200	7.2	16.6	10.5	8.8	16.2	9.2	24.0	18.6

Table 10. Physical and Optical Properties of CRSO Pulp Fraction (>R 80) Produced in CRSO Pilot Plant

Cook Number	1	2	3	4	5	6	7	8
Freeness (mL)	554	410	479	545	272	476	480	481
Bulk Index (cm^3/g)	1.76	1.81	1.59	1.71	2.00	2.36	2.77	2.42
Burst Index (kN/kg)	3.77	4.58	4.56	3.86	3.51	1.77	0.78	1.85
Tear Index ($mN.m^2/g$)	5.68	5.69	6.64	5.64	4.73	4.95	3.36	4.76
Tensile Strength (kN/m)	4.08	4.43	4.29	4.45	4.34	2.55	1.53	2.85
Tensile Energy Adsorption (J/m^2)	64.72	76.41	75.22	63.87	58.76	25.80	9.55	26.14
Stretch (%)	2.45	2.75	2.75	2.33	2.25	1.62	1.03	1.57
Tensile Breaking Length (km)	8.48	7.26	6.77	7.57	7.04	4.40	2.48	4.55
Brightness (%)	32.03	29.95	32.59	31.26	27.43	24.23	26.86	24.00
Opacity (%)	85.33	83.54	80.40	82.94	85.28	92.52	93.74	88.91

Table 11. Comparison of Physical and Optical Properties of Bleached and Non-bleached Pulps Produced from Pilot Plant Run No. 3

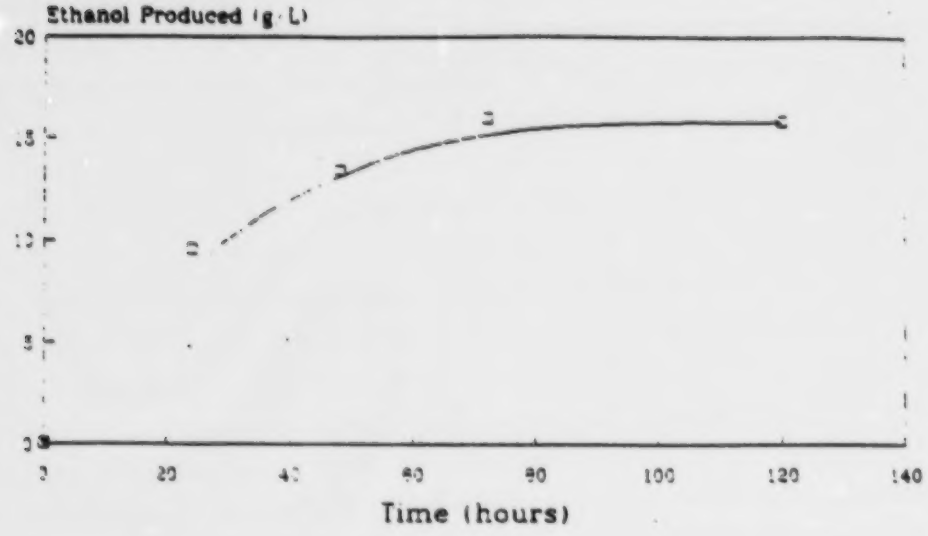
Fraction Bleached	>R 80 No	Total No	>R 80 Yes	>R 48 Yes
Freeness (mL)	497	324	495	558
Bulk Index (cm^3/g)	1.59	1.49	1.35	1.38
Burst Index (MN/kg)	4.58	3.38	2.21	2.02
Tear Index ($mN.m^2/g$)	8.84	4.45	2.18	2.13
Tensile Strength (kN/m)	4.29	4.38	3.18	2.98
Tensile Energy Adsorption (J/m^2)	75.22	58.03	28.48	27.41
Stretch (%)	2.75	2.20	1.54	1.62
Tensile Breaking Length (km)	8.77	7.13	5.39	4.93
Brightness (%)	32.59	29.71	84.30	84.16
Opacity (%)	80.40	87.52	73.78	74.38

The SHF of 10 wt% pulped birch fines (smaller than R 80), pooled from the pilot plant runs to obtain sufficient substrate, produced just over 15 g/L ethanol after 72 h at 30°C (Figure 12). The overall SHF efficiency was significantly lower than that obtained at the bench scale (see Figures 8 and 9). As shown in Figure 13, just over 60% of the potential cellulose was hydrolysed, assuming that the pilot plant fines were 55% cellulose. This figure was not confirmed by separate chemical analysis.

The lower ethanol yield (50%) may be a result of the large head space in the pilot plant fermenter and the poor seal of the top lid. The presence of inadequate anaerobic conditions throughout the fermentation may have led to more yeast growth than expected. Because of the ribbon impeller and internal configuration of the fermenter, it was not possible to obtain complete recovery of the residual solids following SHF. It should be possible to improve this reactor and ensure an anaerobic environment by using a nitrogen blanket and improved seal on the lid.

Figure 12.

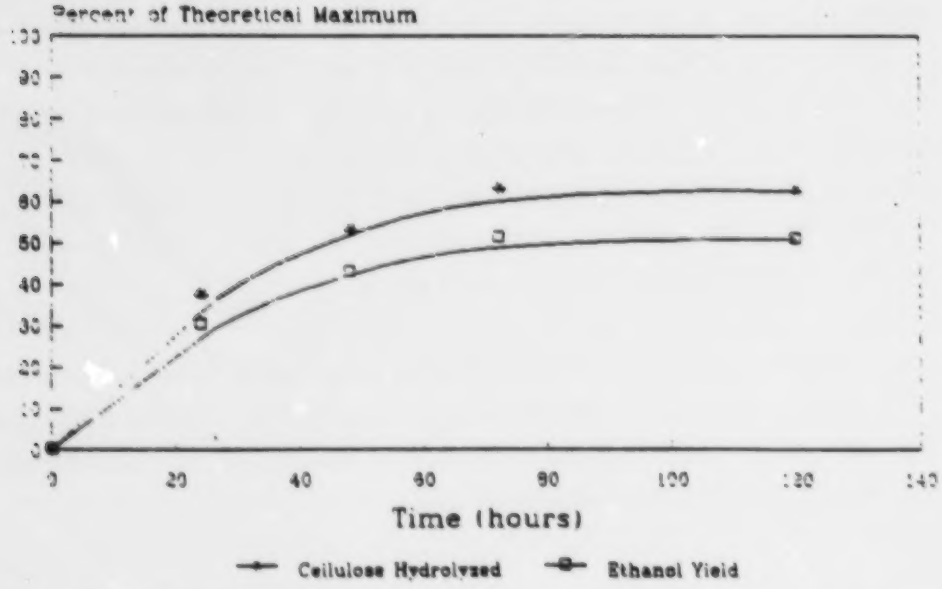
Pilot Plant SHF of 10 wt% Birch Fines



Celluclast, Novozyme and Neutrase used.

Figure 13.

Pilot Plant Efficiency of 10% Fines SHF



Celluclast, Novozyme and Neutrase used

3.7 Economic evaluation of CRSO process

A preliminary economic evaluation of the CRSO process was carried out using a computer model developed at CRSO for this report. Several key parameters were varied using the model to perform sensitivity analyses. A typical printout of the working parameters, material costs and profit calculations for a 600 odt wood per day base case plant is included in Appendix 6.

Table 12 summarizes the effect of plant scale for wood pulping on the production cost and annual volume of 95% (refined) ethanol under the base case conditions when the wood fibre is separated to yield 70% pulp quality fibre and 30% fines for ethanol production. Because the existing distillery at the CRSO has an annual capacity of 27 million litres of refined ethanol, the potential use of adjunct sources of cellulose, specifically, clarifier sludge from local pulp mills (90% cellulose) and waste newspaper (65% cellulose estimated), were included in this analysis to observe the economics when running the distillery at near capacity. The feedstock cost base used in the base case included $65/t odt wood chips and zero cost for wood fines and clarifier sludge.

The direct production cost for ethanol increases with the use of adjunct cellulose sources. This results from the nitric acid pretreatment costs that are not required for the fines fraction from the pulping process. The fines are treated as a waste stream of the pulping process. The best combined process size appears to be a 600 odt/day pulp mill using 55 odt/day clarifier sludge for an adjunct cellulose (fines) source for ethanol. Using the base case pulping and SHF conditions, this combination would allow the distillery to have the lowest direct ethanol production cost, while operating at full capacity. Consequently, this combination was selected for use as the plant scale for subsequent sensitivity analyses.

The effect of wood chip cost on the direct pulp production cost is shown in Figure 14. The base case, using $65/odt of wood chips, gave a pulp cost of $484/odt. From the curve in Figure 14, the pulp production cost varies by $1.79/odt per $1.00 change in the wood chip price.

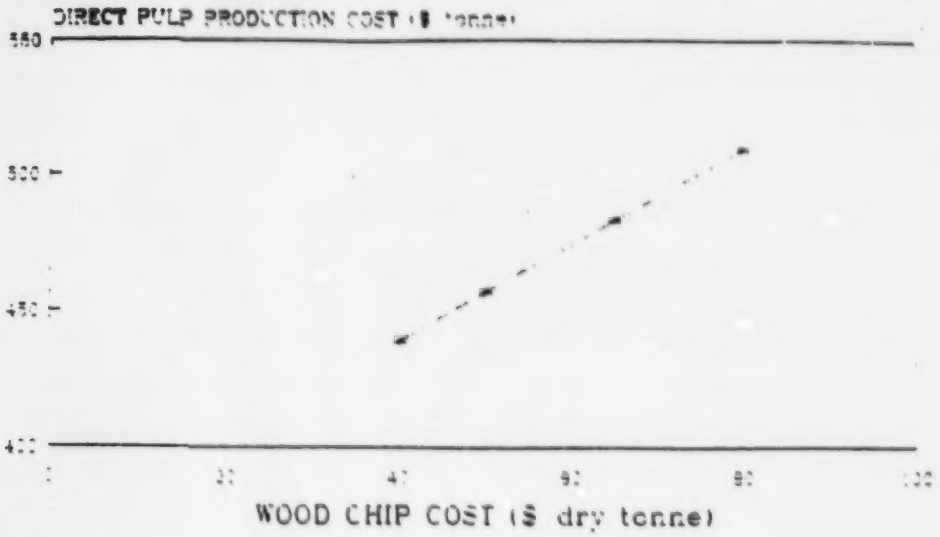
Table 12. Effect of Plant Size and Use of Cellulose Adjuncts on Ethanol Production Cost and Annual Production Volume

Pulp Mill Size (odt wood/day)	Clarifier Sludge (odt/day)	Waste Paper (odt/day)	Ethanol Production Cost (¢/L)	Annual Ethanol Production (10^6 L/year)
400	0	0	47.8	11.9
400	70	30	51.4	26.8
500	0	0	47.2	14.9
500	70	0	49.9	28.3
500	55	25	50.4	28.8
500	0	100	51.7	28.7
600	0	0	48.9	17.9
600	55	0	49.1	28.8
600	40	20	50.4	28.7
600	0	75	49.5	28.7

The effect of nitric acid concentration used in the cooking liquor has a significant effect on direct pulp production cost as shown in Figure 15. This sensitivity in pulp production cost is $28.25/odt per 1 wt% change in nitric acid used in the cooking liquor. The base case used 5.0 wt% nitric acid. The potential for reducing nitric acid concentration is considerable (see Section 3.1). The sensitivity of nitric acid cost on pulp production cost would also be significant, but only one cost ($400/t) was considered in this analysis.

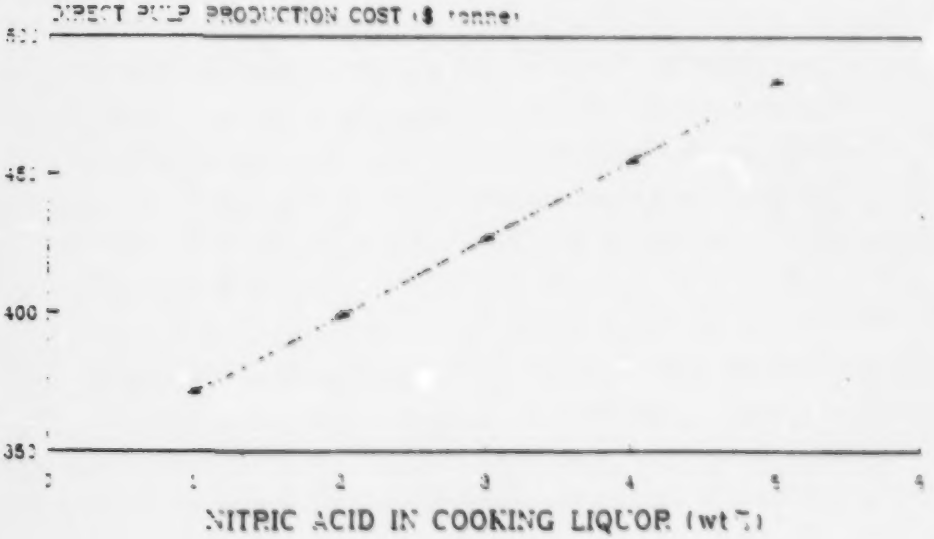
Preliminary studies on recycling or reuse of the cooking liquor after one cook showed that at least 20% of the acid strength could be recovered after removal of the pulped chips. The cooking liquor could be reused, after making it up to full strength with fresh acid, for subsequent cooks. While further work is required to optimize acid recovery and recycle, its effect on pulp production

Figure 14
Effect of Wood Cost on Pulp Cost



600 adt day PLANT - BASE CASE

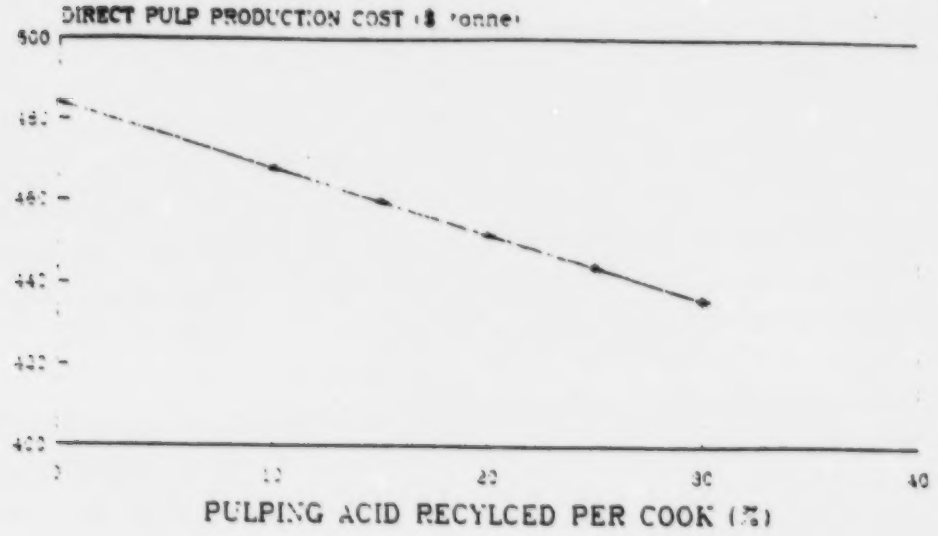
Figure 15
Nitric Acid Concentration vs Pulp Cost



600 adt day PLANT - BASE CASE

Figure 16.

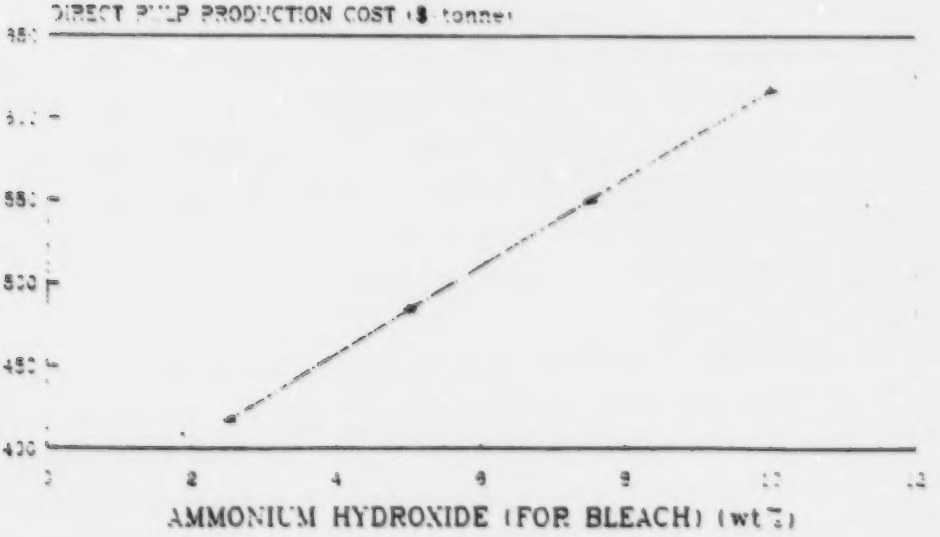
Effect of Recycling Acid on Pulp Cost



600 odt day PLANT - BASE CASE

Figure 17

Bleaching Ammonia Used vs Pulp Cost



600 odt day PLANT - BASE CASE

costs was determined and is shown in Figure 16. The sensitivity is $1.60/odt pulp per 1% recycle of cooking acid (both nitric and phosphoric acids). Other factors, such as less waste acid treatment costs were not considered in this analysis. The base case used zero recycle of cooking acids.

The effect of ammonium hydroxide concentration used in pulp bleaching on the direct pulp production costs is significant as shown in Figure 17. The sensitivity is $26.7/odt per 1% change in ammonium hydroxide bleaching agent. The base case used 5 wt% ammonium hydroxide for bleaching. The sensitivity of ammonia cost was not done, but it would obviously affect pulp production cost in a similar significant fashion.

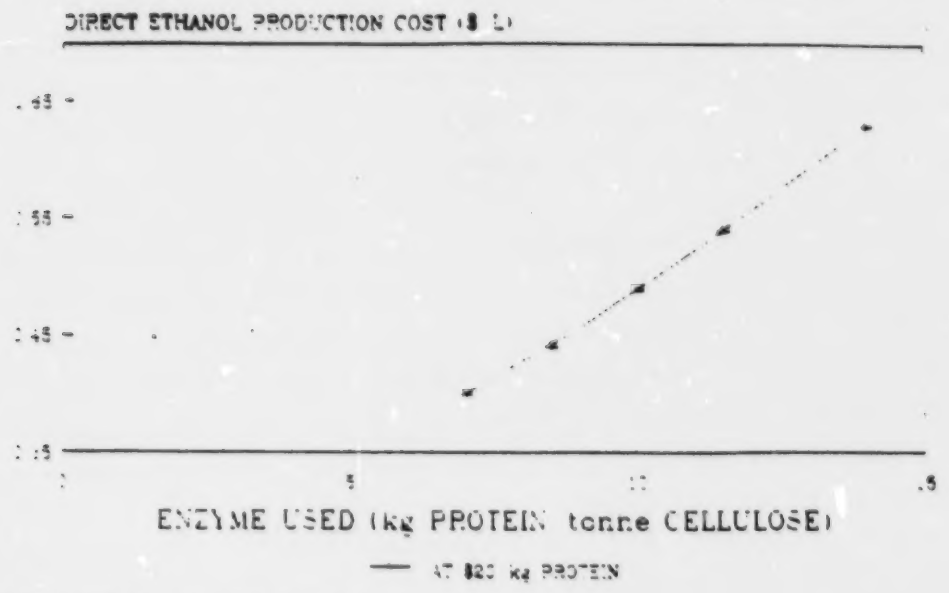
The key factor in ethanol production cost when using a simultaneous hydrolysis and fermentation (SHF) approach is the amount and cost of the cellulase enzymes used. Figure 18 shows the direct ethanol production cost sensitivity on the amount of enzyme (loading) used at the base case price of $20/kg protein. The supplier (ASCAF, Soustons, France) quoted $18/kg based on the annual enzyme bulk requirement (Nativel, 1990). This sensitivity shows that the direct production cost of ethanol varies $0.033/L per kg of enzyme protein/odt cellulose. The base case enzyme loading was 11.5 kg protein/odt of cellulose in the wood fines or approximately 20 MFPU/tonne of wood fines.

Figure 19 shows the direct production cost sensitivity on the cost of enzyme used at the base case loading of 11.5 kg protein/tonne cellulose. The sensitivity shows direct ethanol production cost varies $0.019/L per $1.00 change in the cost per kg of enzyme protein. For example, reducing the base case cost of $20/kg to the latest quoted price of $18 kg/protein would reduce the direct production cost of ethanol by $0.038/L, which translates to a savings of over $1 million in production cost for a 27 million L/year plant.

The use of wood fines in the CRSO pulping process for ethanol production is, in reality, a waste treatment option. For that reason, the actual value of the wood fines should be considered to be zero. The base case used zero value for wood fines and for clarifier sludge and waste paper that could be used as adjunct cellulose sources. If these cellulose sources were used for ethanol

Figure 18.

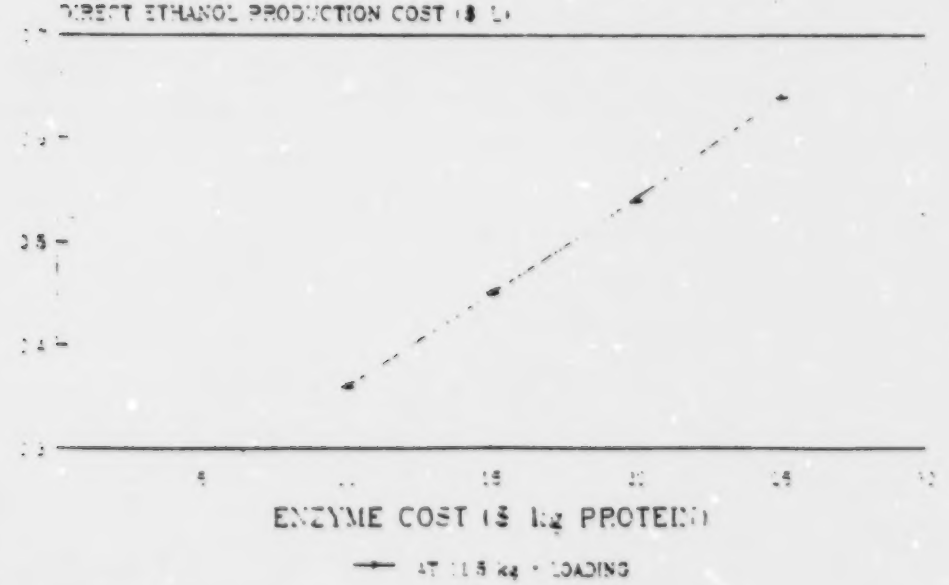
Effect of Enzyme Loading on Ethanol Cost



FOR 800 odt day WOOD, 55 odt day SLUDGE

Figure 19

Effect of Enzyme Cost on Ethanol Cost



FOR 800 odt day WOOD, 55 odt day SLUDGE

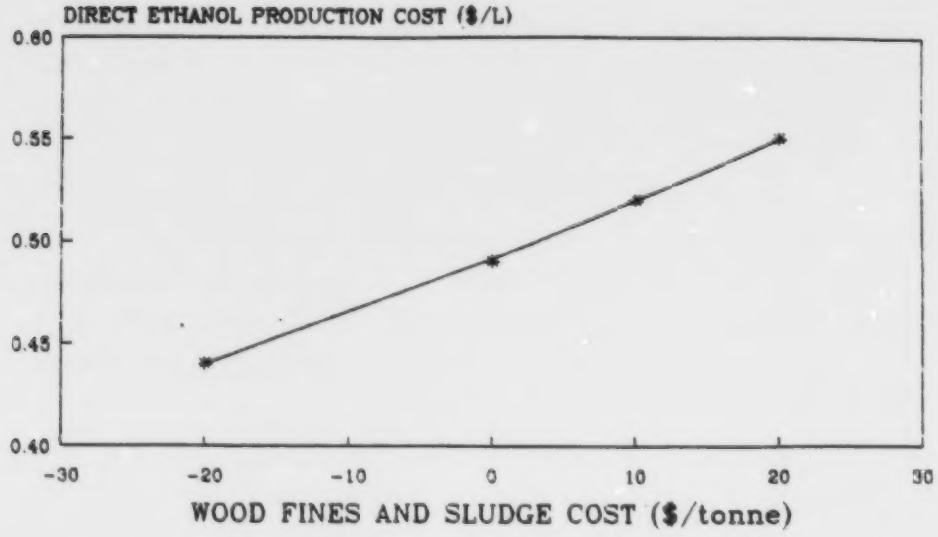
production instead of being disposed of by conventional trucking to land fill disposal sites, we could consider charging a tipping fee (i.e., $20/odt) at the plant, once land fill disposal is no longer an option because of new environmental legislation. The sensitivity of waste cellulose (fibre) cost on the direct ethanol production cost is shown in **Figure 20.** This sensitivity is $0.004/L ethanol per $1.00/odt of waste fibre, which again is significant when considering a 27 million L/year plant.

Other factors to consider in the economic evaluation involve utilities, especially electrical and steam, capital expenditures and other fixed costs. Some preliminary studies were done on sizing plant equipment, water consumption, heating energy requirements and steam requirements for 100, 200 and 600 tonne/day plant scales. These preliminary studies are summarized in Appendix 5. A detailed flow diagram of the CRSO pulping process (wood chips only) can also be found in Appendix 5.

The computer model used to generate the sensitivity analyses described above was also used to determine the return on investment for the base case (600 odt wood/day and 55 odt clarifier sludge/day plant scale) and an improved case in which the cooking liquor nitric acid concentration is decreased from 5 to 4%, acid recycle is 15%, enzyme loading is reduced 25% from 11.5 to 8.5 kg protein/tonne cellulose, and a $20/t tipping fee is charged for the clarifier sludge adjunct. With these feasible improvements, the rate of return improves from 10.7% for the base case to 17.8% for the improved case. Printouts of these scenarios showing all of the parameters considered in the economic evaluation are provided in Appendix 6.

Figure 20.

Waste Cellulose Cost vs Ethanol Cost



600 odt/day WOOD, 55 odt/day SLUDGE

4. CONCLUSIONS

1. The research and development completed during this two year project has resulted in a Canadian patent application (Appendix 1) for a novel catalyzed-nitric acid pulp and ethanol production process applicable to underutilized hardwood species.

2. The pulp characterization tests indicate that the tear index of paper made with 100% CRSO pulp may be too low for use as newsprint furnish, but replacing kraft pulp in newsprint furnish with an optimized birch CRSO pulp is definitely feasible and that there should be a ready market for this pulp.

3. The pulp produced can be used in the unbleached state or it can be bleached to a higher degree of whiteness by extraction with sodium hydroxide or ammonium hydroxide. Chlorine bleaching is not required.

4. Simultaneous hydrolysis and fermentation (SHF) at an initial substrate concentration of 10 to 15 wt%, over 48 to 120 hours allowed 90% cellulose hydrolysis and 73% of the theoretical ethanol yield. These results confirmed that the CRSO nitric acid pulping process renders the cellulose in the pulp fines very susceptible to enzymatic hydrolysis.

5. Waste waters can be treated in an up-flow column reactor containing mycelium of *Scytalidium acidophilum*, an acid-tolerant yeast. Partial removal of xylose, formic acid and acetic acid and colour was demonstrated. With sufficient hydraulic residence time and an adequate bed of active mycelium, more complete removal of these components should be possible to achieve a significant decrease in biochemical oxygen demand (BOD).

6. Preliminary analysis shows the CRSO process to be economically feasible when coupled with the existing ethanol distillery at the Gatineau site.

5. RECOMMENDATIONS

1. A gas incinerator and gas scrubbing system should be designed to treat the reaction gases. Theoretically, after ignition the HCN and NO can be completely oxidized to NO_2, which can then be absorbed in water to form HNO_3 for reuse in the cooking liquor. A feasibility study of this proposed acid recovery process is required.

2. Further studies on minimizing the production of cyanide and optimizing its destruction through hydrogen peroxide oxidation are required. Specifically, the elimination of cyanide in all products and by-products of the CRSO process should be demonstrated.

3. Several approaches to reducing the SHF time for high substrate concentrations should be tested. These include proper mixing of substrate, enzymes and yeast; the use of higher or optimal enzyme loadings; the use of thermotolerant yeasts to allow SHF at temperatures closer to the optimum for enzyme activity (i.e., 45°C instead of 30°C); the use of pH control; and the use of fed-batch SHF to avoid the problems associated with mixing initial high solids.

4. Further research is necessary to define the aeration requirements and residence time required for maximum BOD removal using the acid-tolerant yeast, *Scytalidium acidophilum*. The production of *Scytalidium acidophilum* as an animal feed by-product of this waste treatment system also needs to be demonstrated. The existence of a suitable market for this microbial protein source is also required. Treatability of this wastewater should also be assessed using conventional aerobic treatment technologies.

5. The development of suitable acid-tolerant yeasts for the efficient fermentation of almost 4% xylose in the used cooking liquor should be done to improve the total ethanol yield from the CRSO process.

6. Further research on the disposal or use of the residual SHF solids following distillation is required. This may involve tests as animal feed, composted soil-conditioner or boiler fuel.

7. The use of pulp mill clarifier sludge and recycled waste paper (that portion refused by paper mill deinking plants), as adjunct cellulose sources for ethanol production, should be demonstrated. The use of these substrates may require acid hydrolysis or acid pretreatment before simultaneous enzymatic hydrolysis and fermentation.

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APPENDIX 2.

EXPERIMENTAL DESIGN (BOX-BEHNKEN) FOR FIVE VARIABLES

Run Number (Random Order)	HNO_3 (%)	H_3PO_4 (%)	TEMPERATURE (°C)	TIME (h)	WOOD:ACID RATIO
3	5.0	0.5	80	3	1:4
9	5.0	0.0	80	3	1:4
12	0.5	0.5	80	3	1:4
17	0.5	0.0	80	3	1:4
5	2.5	0.25	95	4	1:4
19	2.5	0.25	95	2.5	1:4
14	2.5	0.25	65	4	1:4
4	2.5	0.25	65	2.5	1:4
10	2.5	0.5	80	3	1:5
13	2.5	0.5	80	3	1:3
1	2.5	0.0	80	3	1:5
18	2.5	0.0	80	3	1:3
8	5.0	0.25	95	3	1:4
20	5.0	0.25	65	3	1:4
6	0.5	0.25	95	3	1:4
21	0.5	0.25	65	3	1:4
15	2.5	0.25	80	4	1:5
22	2.5	0.25	80	4	1:3
2	2.5	0.25	80	2.5	1.5
16	2.5	0.25	80	2.5	1:3
7	2.5	0.25	80	3	1:4
11	2.5	0.25	80	3	1:4
23	2.5	0.25	80	3	1:4
46	2.5	0.5	95	3	1:4
26	2.5	0.5	65	3	1:4
34	2.5	0.0	95	3	1:4
35	2.5	0.0	65	3	1:4
30	5.0	0.25	80	4	1:4
38	5.0	0.25	80	2.5	1:4
27	0.5	0.25	80	4	1:4
39	0.5	0.25	80	2.5	1:4
40	2.5	0.25	95	3	1:5
24	2.5	0.25	95	3	1:3
45	2.5	0.25	65	3	1:5
31	2.5	0.25	65	3	1:3
36	5.0	0.25	80	3	1:5
41	5.0	0.25	80	3	1:3
28	0.5	0.25	80	3	1:5
32	0.5	0.25	80	3	1:3
42	2.5	0.5	80	4	1:4
25	2.5	0.5	80	2.5	1:4
37	2.5	0.0	80	4	1:4
43	2.5	0.0	80	2.5	1:4
33	2.5	0.25	80	3	1:4
29	2.5	0.25	80	3	1:4
44	2.5	0.25	80	3	1:4

APPENDIX 3.

A Rapid Identification Method for Yeasts Producing Ethanol from Xylose or Others Sugars

A draft paper by

Duan Zhi-Zhong

SUMMARY

With this method one can rapidly determine if ethanol is produced from xylose by fermentation. The test was demonstrated on some ethanol producing yeasts. The yeast cells are still viable after the test.

INTRODUCTION

Interest in the production of ethanol from renewable biomass had led to a search for organisms capable of fermenting cellulose derived hexoses and hemicellulose derived pentoses (1-3). A great number of yeast strains have been investigated with reference to their capacity for the assimilation and fermentation of pentose (4). In fermentation research, the most important thing is to know whether ethanol was produced by a new species or a colony of mutants of yeasts. This method was developed to identify yeast strains or mutants which produce ethanol while growing on a sugar-agar plate.

MATERIALS AND METHODS

1. Test solution:

NAD (Nicotinamide Adenine Dinucleotide)	50 mg
NBT (Nitro Blue Tetrazolium)	20 mg
PMS (Phenazine Methosulfate)	2 mg
ADH (Alcohol Dehydrogenase)	3 mg
0.1 M, pH 9.0 Tris-HCl buffer	100 ml

All reagents were obtained from Sigma Chemical Co.

2. For the test, cultures were grown on:

Agar	4 %	
Dextrose	5 %	or
D-xylose	5 %	or
Galactose	5 %	

3. Test organisms:

Scytalidium acidophilum (ATCC 26774), *Pachysolen tannophilus* (NRC 2507) and *Pichia stipitis* (NRC).

The cultures were grown on

Yeast extract	1 %
Difco peptone	2 %
Dextrose	2 %
Bacto agar	1.5 %

4. Test Method:

Yeast cultures were grown 24 hours aerobically, then 24 hours anaerobically. The test solution is then applied with a pipette. Avoid moving the test solution. The plate is then kept in a dark place for 10 minutes. Check under a binocular microscope or with a magnifier for development of colour. If ethanol is produced a violet colour is developed.

RESULTS AND DISCUSSION

	Scyt. acidophilum	*Pa. tannophilus*	*P. stipitis*
Agar only (no sugar)	-	-	-
Glucose (test plate 1)	+	+	+
D-xylose (test plate 2)	-	+	+
Galactose (test plate 3)	-	+	+

After colour testing, the yeast is still viable and can be transferred to another plate. The test is highly specific, and easy to observe. The colour develops in the range of 0.01% to 10% ethanol. There is no interference from methanol, glycerol, acetone, xylitol, acetate.

The principle of this reaction is well known. Ethanol is oxidized by nicotinamide-adenine-dinucleotide (NAD) to acetaldehyde in the presence of the enzyme alcohol-dehydrogenase (ADH).

$$CH_3CHO_2H + NAD \xrightarrow{ADH} CH_3CHO + NADH\ (H^+)$$

The amount of NADH formed in these two reactions is equal to half the amount of ethanol (5-7). Heavy metals and -SH reagent are inhibitors for this reaction (8-11).

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APPENDIX 4.

Fermentation of D-Xylose to Ethanol by the Yeast *Scytalidium acidophilum*

A draft paper by

Duan Zhi-Zhong

SUMMARY

The yeast *Scytalidium acidophilum* was found to be capable of converting D-Xylose to ethanol in the presence of other yeasts. *Pichia stipitis* could not ferment D-xylose to ethanol at pH 2.5, but *Scyt.acidophilum* and *P. stipitis* together could produce ethanol at 6.4 g/L in 11 days, *Pachysolen tannophilus* could produce ethanol at 8.4 g/L in 11 days. When *Scyt. acidophilum* was added, it enhanced the production of ethanol to 10.3 g/L in the same time. Nine combinations of yeasts capable of fermenting D-xylose to ethanol are described.

INTRODUCTION

D-xylose comprises nearly one-third of the reducing sugars obtained from lignocellulose hydrolysis (1). Recently several microorganisms were reported to ferment D-xylose directly to ethanol in significant quantities (25 g/L) and at a yield greater than 0.2 g ethanol per g D-xylose, namely, the yeasts *Pachysolen tannophilus* (2,3), *Pichia stipitis* (4), *Candida species* (5), *Kluyveromyces marxianus* (6) and *Schizosaccharomyces pombe* (7). Several other yeasts also produced ethanol, but at a lower yield (8,9,10). Table 1 shows the yeasts strains investigated with reference to their capacity for the fermentation of D-xylose (11).

A great number of yeasts cannot ferment D-xylose to ethanol (1). *Scytalidium acidophilum* was believed to be one of them. *Scyt. acidophilum* is an acid-tolerant yeast which can ferment at a pH as low as 0.0 (12,13,14). Its mycelium stage can be used for single cell protein production (15) and its yeast stage can ferment hexose to ethanol. In this study we report on the ability of this yeast to ferment D-xylose directly to ethanol.

Table 1. Screening of yeasts for fermentation of xylose (figures in parentheses indicate the number of strains investigated)

Strain	Ethanol ($g\ l^{-1}$)	Xylitol ($g\ l^{-1}$)	Reference
Brettanomyces naardenensis CBS 8042 (11)	1.8		4
Candida guilliermondii (3)	2.5		5
C. shehatae CBS 5813 (6)	6.5		6
C. shehatae CSIR 5701 (27)	20.6		7
C. shehatae Y12866 (3)	24.0	0.2	8
C. sp. 833 (4)	5.8		8
C. tenuis 4436 (11)	6.4		4
C. tenuis CSIR-Y565 (2)	11.0	5.1	7
C. tropicalis KY 5014 (2)	2.8		5
Clavispora sp. (1)	5.9		8
Kluyveromyces sp. KY5188 (1)	4.4		5
K. wickerhamii KY5189 (1)	27.0	22.0	5
Pachysolen tannophilus CBS 4044 (2)	2.1		4
P. tannophilus Y-248050 (1)	16.0		6
Pichia segobiensis CBS 6857 (1)	5.0		4
P. stipitis CBS 5773 (5)	5.9		4
P. stipitis SY-7124 (3)	20.0		6
P. stipitis CSIR-Y633 (2)	22.0		7
Schizosaccharomyces pombe ATCC 20130 (1)	5.0	1.4	8

MATERIALS AND METHODS

Scytalidium acidophilum (ATCC 26774) was obtained from the Agriculture Canada (K.C.Ivarson's) Laboratory. *Pachysolen tannophilus* (NRC 2507) and *Pichia stipitis* (NRC 2548) were obtained from the National Research Council of Canada (NRC) culture collection.

Culture conditions:

Scytalidium acidophilum

PDA agar:	Potato Dextrose Agar	3.9%
	Dextrose	15%
YEMP agar:	Yeast extract	1%
	Malt extract	0.3%
	Difco-Peptone	2%
	Bacto-Agar	2%

Pachysolen tannophilus and *Pichia stipitis*

YEPD agar:	Yeast extract	1%
	Difco-Peptone	2%
	Dextrose	2%
	Bacto-Agar	1.5%

Fermentation Medium:

	g/L
$(NH_4)_2SO_4$	0.5
KH_2PO_4	0.5
Yeast extract	0.5
$MgSO_4.7H_2O$	0.5
$FeSO_4.7H_2O$	0.05
Trace five salts:	
$CuSO4.5H_2O$	
$ZnSO_4.7H_2O$	
$MnCl_2.4H_2O$	
$Na_2MoO_4.2H_2O$	
H_3BO_3	
D-xylose	50

pH adjusted to 2.5

Experimental:

The yeast was harvested from four-day-old culture plates and transferred to the fermentation medium, and the concentration of cells was adjusted to 3 g/L. Five mL (containing 15 mg dry weight yeast cells) were transferred to 500 mL Erlenmeyer flasks containing 200 mL of fermentation media, respectively. The final cell mass concentration of the inocula were 75 mg/L on a dry weight basis. These inocula were shaken at 30°C and 120 rpm.

Yeast Combinations Tested:

No.	Yeast (dry wt)
1.	15 mg *Scytalidium acidophilum* (grown on PDA)
2.	15 mg *Scyt. acidophilum* (grown on YMP agar)
3.	15 mg *Pachysolen tannophilus*
4.	15 mg *Pichia stipitis*
5.	7.5 mg *Scyt. acidophilum* + 7.5 mg *Pa. tannophilus*
6.	7.5 mg *Scyt. acidophilum* + 7.5 mg *Pi. stipitis*
7.	6 mg *Scyt. acidophilum* + 6 mg *Pa.tannophilus* + 6 mg *Pi. stipitis*
8.	7.5 mg *Pa. tannophilus* + 7.5 mg. *Pi. stipitis*
9.	15 mg *Scyt. acidophilum* + 2 g D-glucose

Analytical:

D-xylose concentration was measured with a Waters ALC-201 HPLC. Ethanol and xylitol were measured with the same HPLC, the column was a Brownlee Labs 2014. Biomass concentration was measured with a LKB 4049-900 Novaspec spectrophotometer at 600 nm.

RESULTS AND DISCUSSION

Pichia stipitis alone did not ferment D-xylose to ethanol at pH 2.5. *Scytalidium acidophilum* (grown on YMP agar) did not ferment D-xylose to ethanol either. But the two microorganisms together could ferment D-xylose to ethanol at 6.39 g/L in 11 days.

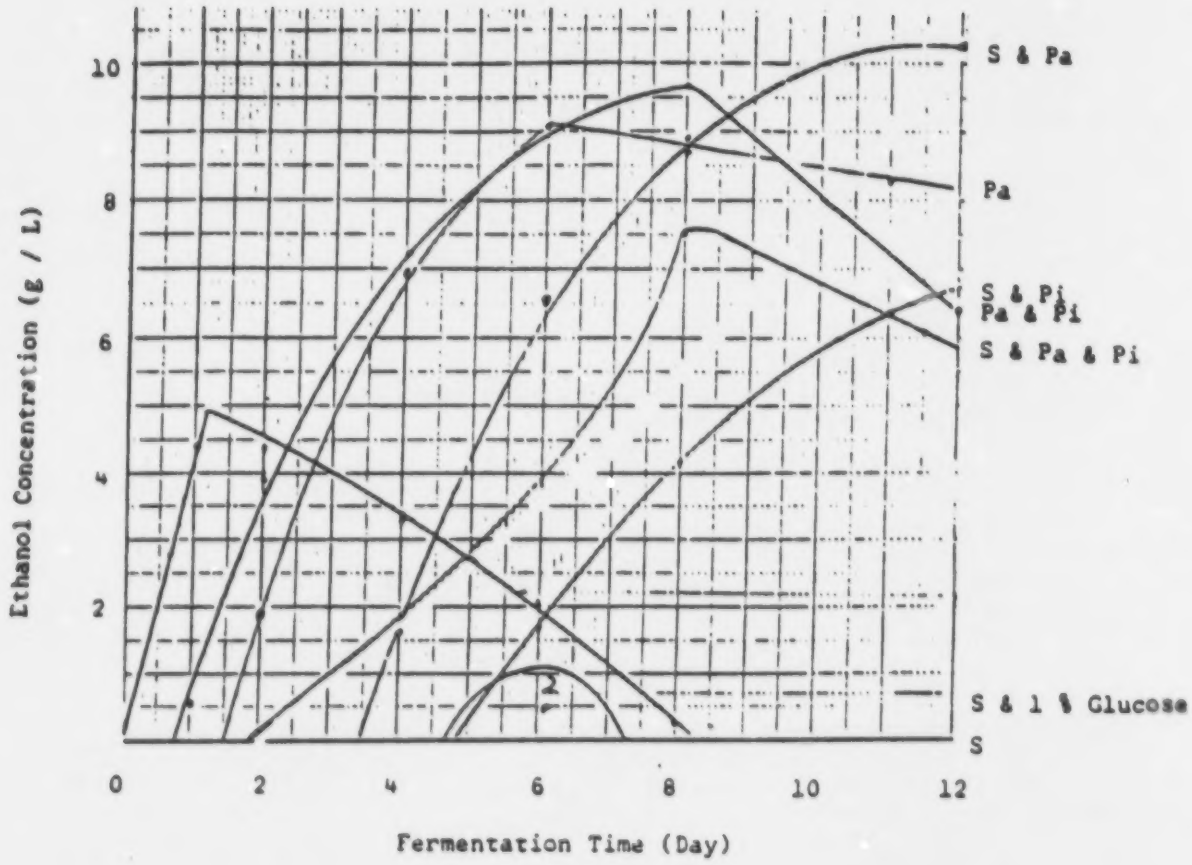
Figure 1 shows the course of the ethanol production from 50 g/L D-xylose at pH 2.5. *Scytalidium acidophilum* combined with *Pachysolen tannophilus* produced more ethanol (10.28 g/L) than *Pachysolen tannophilus* alone (8.33 g/L) in 11 days.

C.S. Gong *et al.* (16) observed that many yeasts quantitatively metabolize D-xylose if converted to D-xylulose. The xylulose is phosphorylated to xylulose-5-phosphate and channelled into the pentose-phosphate pathway to produce pyruvate as the key intermediate.

Bruinenberg *et al.* (17) had suggested that the poor fermentation of D-xylose by *P. tannophilus* is due to the enzyme xylose-reductase which nearly exclusively uses NADPH as coenzyme, so that by the reductive/oxidative xylose pathway a surplus of NADH is produced which blocks other metabolic activities. In the meantime, these authors were able to show that in *Pichia stipitis*, xylose reductase activity is linked not only to NADPH but also to NADH, and they correlated this result with the better xylose fermentation capability (18).

Figure 2 shows the time course of D-xylose utilization at pH 2.5. Figure 3 shows the time (days) course of xylitol production at pH 2.5. Xylitol was a major by-product when D-xylose was fermented to ethanol by some yeasts. In general, xylitol was not a good substrate when compared to either D-xylose or D-xylulose (19). *Pachysolen tannophilus* metabolizes xylitol very slowly and only under aerobic conditions (20). Ethanol yields are reduced when xylose is shunted to xylitol. It was thought that xylitol accumulation could be minimized by using a lower initial xylose concentration (20g/L), followed by a slow addition of D-xylose (1.6 g D-xylose/h) after 24 h (20). According to Maleszka (8), *Pachysolen tannophilus* can convert more than 15% of the initial D-xylose concentration to the low ethanol yield found with *Candida shehatae*.

Figure 4 shows the time (days) course of biomass production D-xylose at pH 2.5. It should be noted that in many papers, the productivities were not related to cell mass number. Slininger (3) suggested that the final cell mass concentration of the inocula were 3 g/L on a dry weight basis. In this experiment, the final cell mass concentration of the inocula were 0.075 g/L on a dry weight basis.

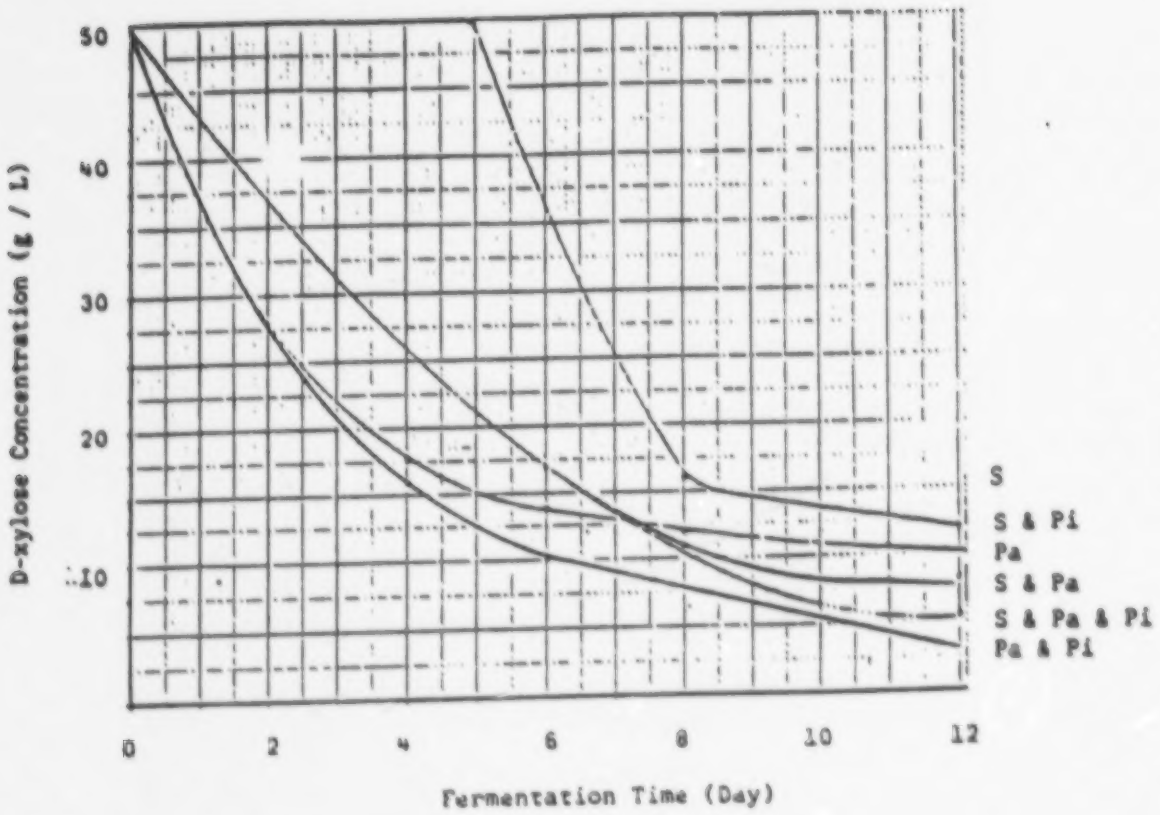


S: Scytalidium acidophilum

Pa: Pachysolen tannophilus

Pi: Pichia stipitis

Figure 1: Ethanol production from 5% D-xylose by different Microorganisms at pH 2.5

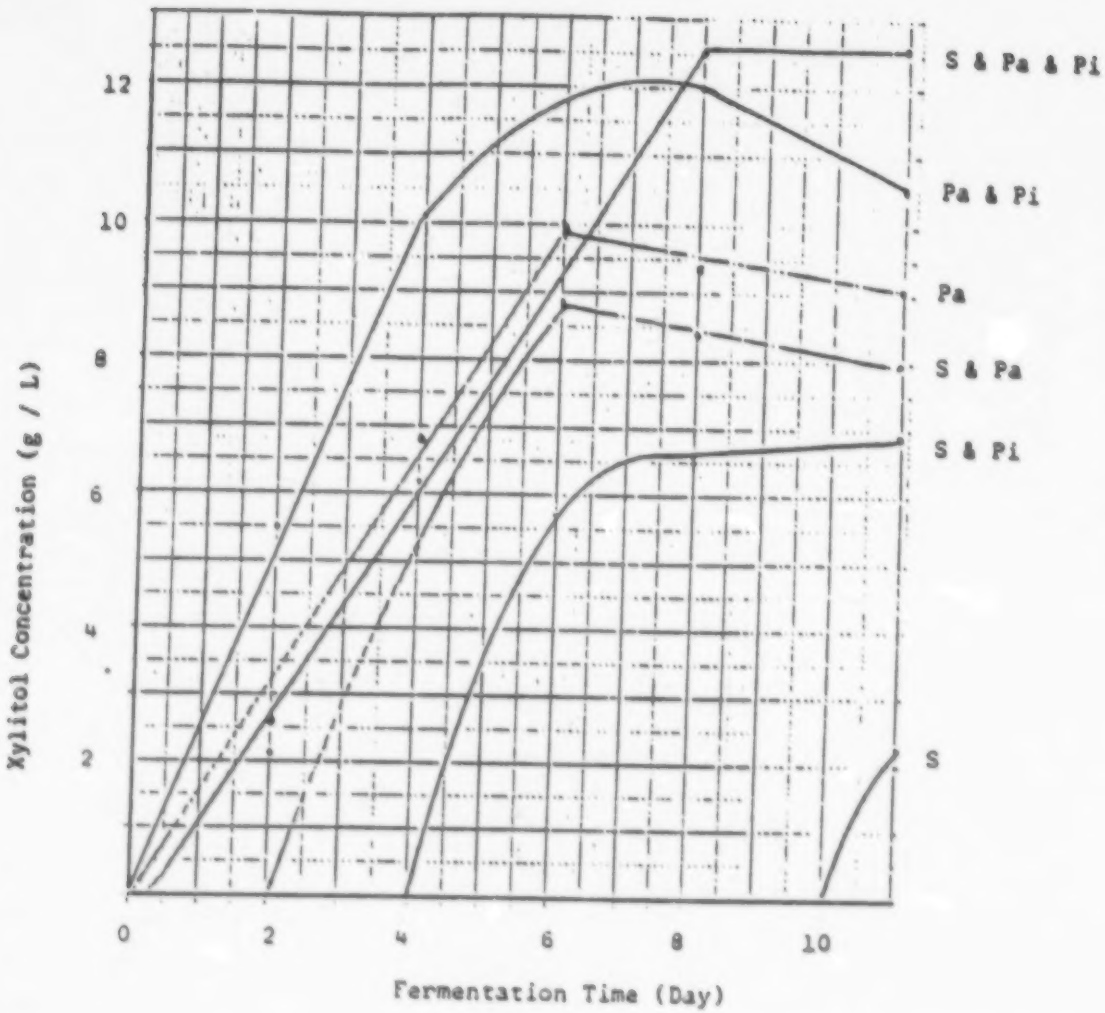


S: Scytalidium acidophilum

Pa: Pachysolen tannophilum

Pi: Pichia stipitis

Figure 2: D-xylose utilization at pH 2.5

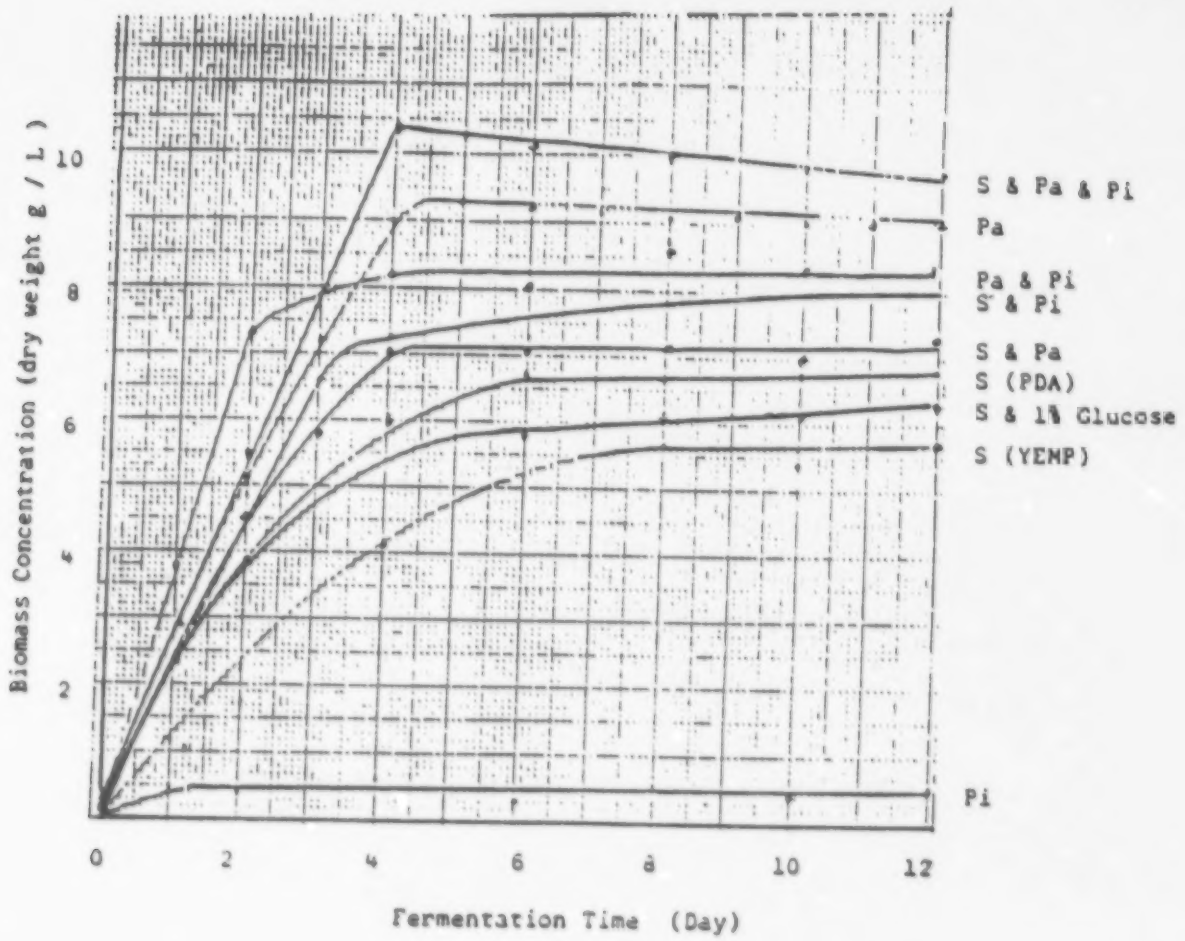


S: Scytalidium acidophilum

Pa: Pachysolen tannophilus

Pi: Pichia stipitis

Figure 3 : Xylitol production from 5% D-xylose by different Microorganisms at pH 2.5



S: Scytalidium acidophilum

Pa: Pachysolen tannophilus

Pi: Pichia stipitis

Figure 4: Biomass production from 5% D-xylose by different Microorganisms at pH 2.5

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APPENDIX 5

PRELIMINARY ENGINEERING DESIGN STUDY FOR CRSO PULPING PROCESS

Veronica Rojas Paez
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INTRODUCTION

This report provides preliminary design data on the size of equipment, quantity of input materials and the steam consumption for the production of pulp and ethanol from birch wood chips by the CRSO process. These calculations were based on a pulp mill treating 100 bone dry tonnes (bdt)/day of wood chips and extrapolated to plant sizes of 200 and 600 bdt/day.

The cooking yields (yield of total wood fibre after pulping) were determined experimentally in the CRSO pilot plant to be between 68 and 99 wt% of the input dry wood weight. The range of cooking yield reflects the different cooking conditions and desired quality of the pulp produced. The following calculations were based on a cooking yield of 90% and an after bleaching yield of 90% for an effective pulp fibre yield of 81%. Equipment capacity estimates used a plus 20% error margin.

The primary process operation parameters used in these calculations included the following:

- The chemi-mechanical pulping process is operated on a discontinuous basis with 6 cooks/day.

- The wood chips are impregnated with cooking liquor under vacuum (635 mm Hg) before going to the digester.

- The cooking liquor consists of nitric acid and phosphoric acid.

- The cooking liquor is used twice and is then neutralized with spent bleaching agent. After concentration, these residues are sent to a furnace for incineration.

- The combined process water from the cooked chip washing, refining and screening stages is recycled for these operations.

- The pulped cooked wood chips are fiberized using disk refiners and the fibres separated into 70% long fibres and 30% short fibres (fines).

- The short fibres are used for ethanol production by a simultaneous enzymatic hydrolysis and fermentation (SHF) process.

- The long fibres are used for pulp, either unbleached or bleached.

- The bleaching of the pulp is done in two stages: a secondary cooking treatment with nitric acid, followed by delignification using either ammonium hydroxide or sodium hydroxide.

The calculations have been summarized in the following five tables. In order to have an overview of the whole process, a flow diagram is presented in Figure 1.

Table 1. Equipment Capacity (m^3)

Number[1]	Description	Plant Capacity (bdt/day) 100	200	600
7	Used water reservoir	280	560	560
8	Long fibre holding tank	84	168	168
9	Short fibre holding tank	324	648	972
10	Digester	111	222	222 (3)[2]
11	Acid impregnation vacuum tank	111	222	222
15	Used acid reservoir	101	202	202
16	2° Cooking reactor (pre-bleaching)	134	268	268
17	Ammonium hydroxide reservoir	26	52	52
21	Used cooking liquor reservoir	10 (2)	20 (2)	20 (2)
22	Fermenter (SHF)	325 (3)	650 (3)	975 (6)
28	Wood chip silo	105	210	210
30	Cooking liquor reservoir	67	134	134
37	Used water tank	72	144	144
39	Used water tank	91	182	182
41	Pulp washing tank	200	400	400
45	Used water tank (1st, 2nd wash)	240	480	480
46	Enzyme reservoir	2	4	6
48	Used water tank (3rd wash)	200	400	400
51	Filter (chip/liquor separator)	133	266	266
52	Pulp holding tank	204	408	408
53	Bleaching reactor	65	130	130
60	Used ammonium hydroxide reservoir	24	48	48
62	Neutralizing tank	138	276	276

(1) Numbers refers to Figure 1, process flow diagram.
(2) Number of vessels required.

Table 2. Water Consumption

Utilization	Location	m^3/cook	m^3/tonne[1]	m^3/100 tonnes
Cooking liquors	Cooking liquor reservoir (30)	35	2.09	209
Chip washing	Filter (51)	60	3.6	360
Pulp wash after 2° cook	Long fibre press (18)	76	4.56	456
Ammonium hydroxide solution	Ammonium hydroxide reservoir (17)	17	1.02	102
Pulp wash after bleaching	Pulp washing tank (41)	315	18.9	1890
TOTAL WATER		503	30.17	3017

(1) Bone dry tonne of wood chips.

Table 3. Heating Energy Requirement Per Cook in Digester

Item	Weight (kg)	Specific Heat (kcal/kg. °C)	Change in Temperature from	to	increase	Energy Required (Q) $\times 10^3$ kcal
Steel digester	35,000	0.12	75	95	20	0.84
Ceramic insulation	4,000	0.20	50	55	5	0.04
Dry wood chips	16,700	0.33	15	95	80	4.41
Water in wood chips	11,100	1.00	15	95	80	8.88
Fresh cooking liquor	39,000	0.95	15	95	80	29.64
Used cooking liquor	4,200	0.98	90	95	5	0.20
Wash water	11,100	0.98	52	95	43	4.68
Nitric acid (83%) + Phosphoric acid (85%)	1,400	0.63	15	95	80	0.71

Total energy required per cook = IQ = 4.94×10^6 kcal.
Additional 10% added for heat loss gives total estimate of 5.43×10^6 kcal.

Table 4. Heating Energy Requirement Per Secondary Cooking Reactor

Item	Weight (kg)	Specific Heat (kcal/kg. °C)	Change in Temperature from	to	increase	Energy Required (Q) $\times 10^3$ kcal
Steel reactor	43,000	0.12	70	90	20	1.03
Ceramic insulation	4,900	0.20	50	55	5	0.05
Dry pulp fibre	10,500	0.33	20	90	70	2.43
Water in pulp	59,500	1.00	20	90	70	41.65
Recycled liquor	42,000	0.95	85	90	5	2.02
Nitric acid (83%)	3,700	0.63	15	90	75	1.75

Total energy required per cook = IQ = 4.89×10^6 kcal.
Additional 10% added for heat loss gives total estimate of 5.38×10^6 kcal.

Table 5. Comparison of Steam Consumption for Process With or Without Recycling of Cooking Liquor and Process Water

	Steam consumption per bone dry tonne wood	
	Primary Cook	Secondary Cook
With Recycling	721 kg	1000 kg
Without Recycling	668 kg	662 kg
Steam consumption reduction by recycle	7.4%	34.3%

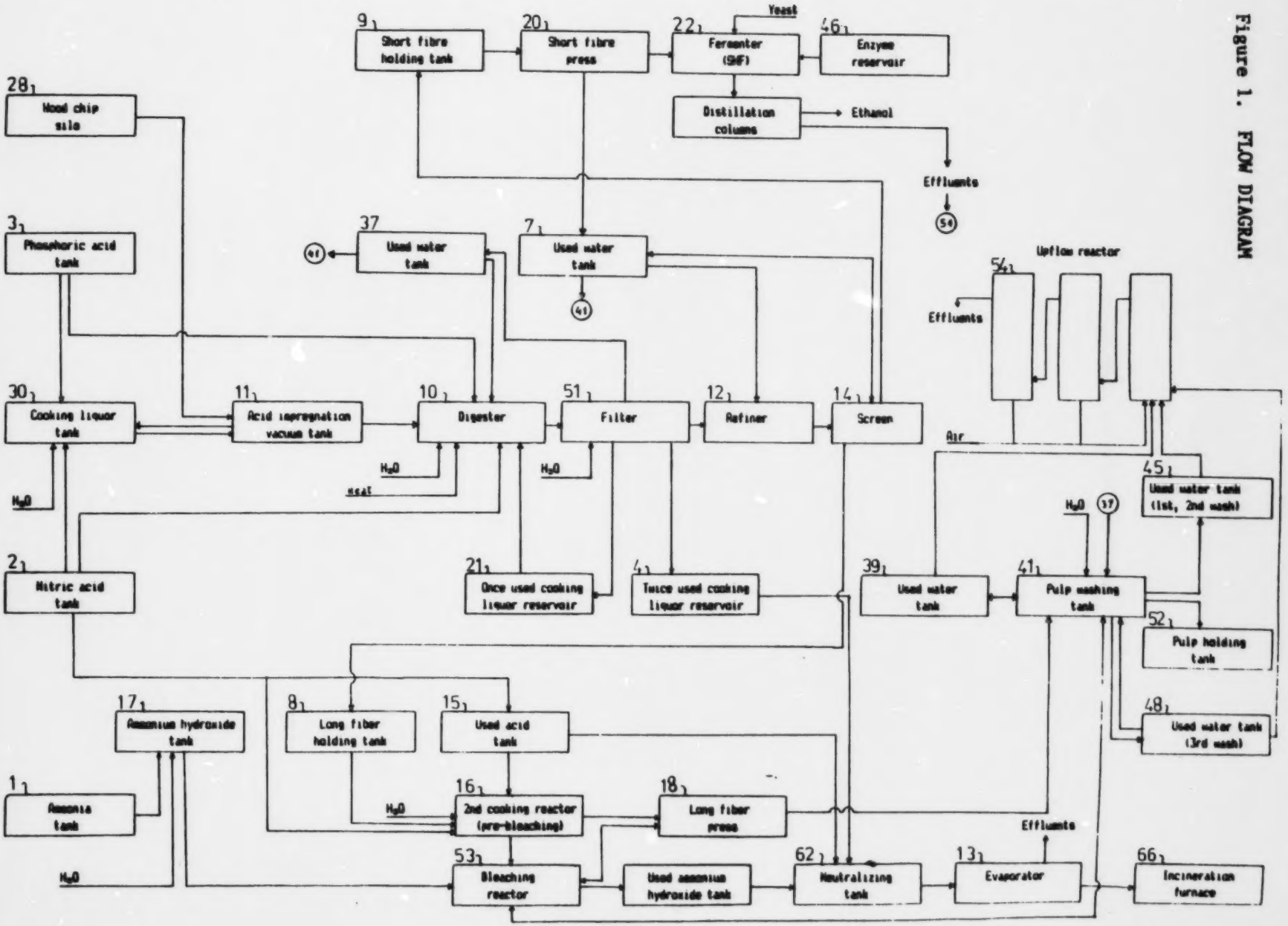

Figure 1. FLOW DIAGRAM

APPENDIX 6

PRELIMINARY ECONOMIC ANALYSIS

PRELIMINARY ECONOMIC EVALUATION OF ORGANOSOLV PULPING PROCESSES USING CATTAIL DISTILLERY (BASE CASE)

Process Parameters:	Input value	Units	Process Parameters:	Input value	Units
Plant capacity (wood)	600	odt/day	Cooking temperature	95	degrees C
Plant capacity (wastepaper)	0	odt/day	Cooking time	3	hours
Plant capacity (sludge)	55	odt/day	Total wood fibre yield	90	wt% of odt wood
Plant operation	330	days/yr	After bleaching yield	90	wt% of odt fibre
Annual Capacity (wood)	198,000	odt/yr	Pulp yield from wood fibre	70	wt%
Annual Capacity (wastepaper)	0	odt/yr	Fines yield from wood fibre	20	wt%
Annual Capacity (sludge)	18,150	odt/yr	Fines yield from wastepaper	90	wt%
Distillation capacity	27,000,000	L/yr	Fines yield from clarifier sludge	90	wt%
Capital Cost	125,000	$/t-day	Fermentation yield	0.48	g ethanol/g glucose
Capital Cost	3e9.8e	$/t-yr	Cellulose in fines	55	wt%
Total Investment	$1,300,000	$ Can.	Cellulose in wastepaper	65	wt%
Plant Service life	10	years	Cellulose in clarifier sludge	90	wt%
Income Tax Rate	50	%	Enzyme loading (ASCAF cellulases)	72	L/tonne cellulose
moodification ratio	0.25		Enzyme activity (FPU)	0.20	nFPU/L
Nitric acid (pulping)	5.0	wt%	Protein in ASCAF cellulase prep.	9.1e	kg/L
Nitric acid (pre-bleach	3.0	wt%	Total enzyme protein used	41.5	kg/tonne cellulose
Nitric acid (paper sludge)	2.0	wt%	Cellulose hydrolyzed in SHF	90	wt%
Phosphoric acid conc.	0.25	wt%	Bleaching ammonia conc.	5	wt%
Total cooking acid recycle	0	wt%	Neutralization ammonia conc.	0.2	wt%

All $ tonne-year figures based on input tonnes of wood and adjunct cellulose sources

DIRECT PRODUCTION COSTS:	For Pulp	For Ethanol	Units	$/unit	Pulp Costs Annual	Pulp Costs $/tonne	Ethanol Costs Annual	Ethanol Costs $/L
Wood Chips	198,000	0	od tonne	65.00	$12,870,000	$114.64	$0	$0.000
Wood Chip Fines (waste)	0	52460	od tonne	0.00	$0	$0.00	$0	$0.000
Waste paper	0	0	od tonne	0.00	$0	$0.00	$0	$0.000
Clarifier sludge	0	18,150	od tonne	0.00	$0	$0.00	$0	$0.000
Nitric acid (bulk,bleaching)	5e,5e9	1,452	tonne	400.00	$21,827,520	$194.43	$520,300	$0.022
Phosphoric acid	1,980	202	tonne	1050.00	$2,079,000	$18.52	$190,575	$0.007
Ammonia (bleach, neutralize)	e9,89e	572	tonne	300.00	$14,968,000	$133.33	$171,864	$0.006
Cellulases (ASCAF)	0	508,094	mg protein	20.00	$0	$0.00	$10,161,677	$0.379
Electricity	19,800,000	13,741,560	kWh	0.024	$475,200	$4.23	$257,796	$0.010
Steam (plus distillation)	396,000	372,e96	GJ	4.00	$1,584,000	$14.11	$1,489,184	$0.056
Water	12,27e,300	4,e39,820	cubic metres	0.02	$245,520	$2.19	$88,796	$0.003
Labour	6	6	man/years	40000.00	$240,000	$2.14	$240,000	$0.009
Total Direct Production Costs:								
TOTAL DIRECT PRODUCTION COSTS ($/tonne-yr):		312.15	(Pulp + Ethanol)		54,290,840	$483.58	$3,:80,692	$0.491
FIXED COSTS:								
Depreciation @ 10% C.I.	90	10	% of total	% Cap.Invst.	$7,290,000	$64.94	$810,000	$0.030
Tax and Insurance @ 2% C.I.	75	25	% of total	% Cap.Invst.	$e07,500	$5.41	$202,500	$0.008
Finance Int. @ 10% of C.I.	90	10	% of total	% Cap.Invst.	$7,290,000	$64.94	$810,000	$0.030
PLANT OVERHEAD COSTS:	100	100		% of Labour	$240,000	$2.14	$240,000	$0.009
Total Production Costs:								
TOTAL PRODUCTION COSTS ($/tonne-year):		393.06	(Pulp + Ethanol)		$e9,717,5e0	$421.00	$15,243,192	$0.5e8
REVENUE:								
Bleached Pulp	112,266	0	tonne	700.00	$78,586,200		$0	
Ethanol (Refined) FOB Plant	0	2e,828,952	L	0.62	$0		$16,633,950	
Ethanol (Crude) FOB Plant	0	0	L	0.32	$0		$0	
Animal Feed/Compost etc.	0	2,117	tonne	150.00	$0		$317,521	
Total Revenue:								
TOTAL REVENUE ($/tonne-year):		442.00	(Pulp + Ethanol)		$78,586,200		$16,951,471	
Taxable Income:								
TAXABLE INCOME $ tonne-year):		48.92	(Pulp + Ethanol)		$8,868,660	$79.00	$1,708,278	$0.064
Net Profit:								
NET PROFIT $/tonne-year):		24 e7	(Pulp + Ethanol)		$4,434,330	$39.50	$854,139	$0.032
CASH FLOW (Net Profit + Depreciation) ($/t-yr):			81.94					
PRESENT WORTH FACTOR:			5.97					
PW CASH FLOW ($/tonne):			3e9.73					
INITIAL INVESTMENT ($/tonne):			3e9.8e					
PAYOUT PERIOD (years):			5.97					
RATE OF RETURN (%/yr):			10.e8					

PRELIMINARY ECONOMIC EVALUATION OF ZEDO NITRIC ACID PULPING PROCESS USING BATCHEAU DISTILLERY (IMPROVED CASE 4)

Process Parameters:	Input Value	Units	Process Parameters:	Input Value	Units
Plant Capacity (wood)	600	odt/day	Cooking temperature	95	degrees C
Plant Capacity (wastepaper)	0	odt/day	Cooking time	3	hours
Plant Capacity (sludge)	55	odt/day	Total wood fibre yield	90	wt% of odt wood
Plant Operation	330	days/yr	After Bleaching yield	90	wt% of odt fibre
Annual Capacity (wood)	198,000	odt/yr	Pulp yield from wood fibre	70	wt%
Annual Capacity (wastepaper)	0	odt/yr	Fines yield from wood fibre	30	wt%
Annual Capacity (sludge	18,150	odt/yr	Fines yield from wastepaper	90	wt%
Distillation capacity	27,000,000	L/yr	Fines yield from clarifier sludge	90	wt%
Capital Cost	133,000	$/tpd-day	Fermentation yield	0.48	g ethanol/g glucose
Capital Cost	369.8%	$/t-yr	Cellulose in fines	55	wt%
Total Investment	$4,000,000	$ Can.	Cellulose in wastepaper	65	wt%
Plant Service Life	10	years	Cellulose in clarifier sludge	90	wt%
Income Tax Rate	50	%	Enzyme loading (ASCAF cellulases)	53	L/tonne cellulose
Wood:liquor ratio	0.25		Enzyme activity (FPU)	0.30	nFPU/L
Nitric acid (pulping,	4.0	wt%	Protein in ASCAF cellulase prep.	0.16	kg/L
nitric acid (pre-bleach)	1.0	wt%	Total enzyme protein used	8.5	kg/tonne cellulose
Nitric acid (paper,sludge)	1.0	wt%	Cellulose hydrolyzed in SHF	90	wt%
Phosphoric acid conc	0.25	wt%	Bleaching ammonia conc.	5	wt%
Total cooking acid recycle	15	wt%	Neutralization ammonia conc.	0.2	wt%

All $ tonne-year figures based on input tonnes of wood and adjunct cellulose sources

DIRECT PRODUCTION COSTS.	For Pulp	For Ethanol	Units	$/unit	Pulp Costs Annual	Pulp Costs $/tonne	Ethanol Costs Annual	Ethanol Costs $/L
Wood Chips	198,000	0	od tonne	65.00	$12,870,000	$114.64	$0	$0.000
Wood Chip Fines (waste)	0	53,460	od tonne	0.00	$0	$0.00	$0	$0.000
Waste paper	0	0	od tonne	-20.00	$0	$0.00	$0	$0.000
Clarifier sludge	0	18,153	od tonne	-20.00	$0	$0.00	($363,060)	($0.014)
nitric acid (pulp,bleaching)	41,847	4,224	tonne	400.00	$16,738,720	$149.28	$649,680	$0.018
Phosphoric acid	1,683	454	tonne	1050.00	$1,767,150	$15.76	$161,989	$0.006
Ammonia (bleach, neutralize)	49,896	573	tonne	300.00	$14,968,800	$133.33	$171,864	$0.006
Cellulases ASCAF	0	376,006	kg protein	20.30	$0	$0.00	$7,680,123	$0.279
Electricity	19,800,000	10,741,500	kwh	0.024	$475,200	$4.23	$257,796	$0.010
Steam (plus distillation)	396,000	372,296	GJ	4.00	$1,584,000	$14.11	$1,489,184	$0.056
Water	12,276,000	4,439,820	cubic metres	0.02	$245,520	$2.19	$88,796	$0.003
Labour	0	0	man,years	40000.00	$240,000	$2.14	$240,000	$0.009
Total Direct Production Costs:					48,909,390	$435.66	10,020,432	$0.373
TOTAL DIRECT PRODUCTION COSTS ($/tonne-yr):		272.62	Pulp + Ethanol)					
FIXED COSTS:								
Depreciation @ 12% C.I.	90	10	% of total	% Cap.Invst.	$7,290,000	$64.94	$810,000	$0.030
Tax and Insurance @ 1% C.I.	75	25	% of total	% Cap.Invst.	$607,500	$5.41	$202,500	$0.008
Finance Int. @ 12% of C.I.	90	10	% of total	% Cap Invst.	$7,290,000	$64.94	$810,000	$0.030
PLANT OVERHEAD COSTS.	100	100	% of Labour		$240,000	$2.14	$240,000	$0.009
Total Production Costs:					$64,336,890	$572.88	$12,082,932	$0.450
TOTAL PRODUCTION COSTS ($/tonne-year):		353.55	(Pulp + Ethanol)					
REVENUE:								
Bleached Pulp	412,266	0	tonne	700.00	$79,586,200		$0	
Ethanol (Refined) FOB Plant	0	26,828,952	L	0.62	$0		$16,633,950	
Ethanol (Crude) FOB Plant	0	0	L	0.32	$0		$0	
Animal Feed/Compost etc.	0	2,117	tonne	150.00	$0		$317,521	
Total Revenue:					$79,586,200		$16,951,471	
TOTAL REVENUE ($/tonne-year):		442.00	(Pulp + Ethanol)					
Taxable Income:					$14,249,310	$126.92	$4,868,538	$0.181
TAXABLE INCOME ($/tonne-year):		88.45	(Pulp + Ethanol)					
Net Profit:					$7,124,655	$63.46	$2,434,269	$0.091
NET PROFIT ($/tonne-year)		44.22	Pulp + Ethanol)					

CASH FLOW (net profit + Depreciation, $/t-yr):	$1.70
PRESENT WORTH FACTOR:	4.53
PW CASH FLOW ($/tonne):	$69.78
INITIAL INVESTMENT ($/tonne):	$69.84
PAYOUT PERIOD (years):	4.53
RATE OF RETURN (%/yr):	17.83